2008

Aspects of the biology and conservation of the endangered Oxleyan pygmy perch *Nannoperca oxleyana* Whitley

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*Southern Cross University*

Publication details

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ASPECTS OF THE BIOLOGY AND CONSERVATION
OF THE ENDANGERED OXLEYAN PYGMY PERCH
NANNOPERCA OXLEYANA WHITLEY

SUBMITTED BY
JAMES T. KNIGHT (B. Appl. Sc. Hons.)

Nannoperca oxleyana
(mature female, 50mm TL)

A thesis prepared in total fulfilment of the requirements for the degree of
Doctor of Philosophy

School of Environmental Science and Management
Southern Cross University

2008
DECLARATION

I declare that this submission is my own work and that, to the best of my knowledge it contains no material written by another person, nor material which has been submitted for a higher degree to Southern Cross University or any other institution, except where due acknowledgement has been made in the text.

James T. Knight
August 2008
To Carol Knight
who instilled in me a deep appreciation for nature.
May she rest in peace.
SUMMARY

The endangered Oxleyan pygmy perch *Nannoperca oxleyana* Whitley is a small-bodied freshwater percichthyid fish endemic to coastal mid-eastern Australia. In this study, several poorly understood aspects of the species’ biology were described and related to the ways that environmental variables and anthropogenic impacts may have influenced current patterns of distribution and abundance. Using this information, a number of recovery-based management and research principles for the conservation of the species were developed.

Initially, a standardised, non-destructive sampling protocol was designed that effectively and efficiently detected the presence, and quantified the relative abundance, of extant populations of *N. oxleyana*. Through evaluation of field experiments and the analysis of survey data a protocol was recommended that includes saturating sites with unbaited traps set for at least 30 minutes and sampling with a backpack electrofisher. It was determined that seine netting should be reserved for situations where an electrofisher is unavailable or non-deployable.

Using this protocol, the distribution and habitat associations of *N. oxleyana* in south-eastern Queensland and north-eastern New South Wales (NSW) were documented. The species’ range encompasses approximately 530 km of coastline from Coongul Creek on Fraser Island, Queensland (25° 16’S, 153° 09’E) south to Tick Gate Swamp near the township of Wooli, NSW (29° 54’S, 153° 15’E). It is confined primarily to dystrophic, acidic, freshwater systems draining through sandy coastal lowlands and *Banksia*-dominated heath ecosystems. Both lentic and lotic environments provide habitat for the species but it is found only in slow flowing pools and backwaters of river channels and tributaries as well as in swampy drainages, lakes, ponds and dams. Beds of emergent or submerged plants, steep/undercut banks fringed with the semi-submerged riparian vegetation, leaf litter and snags were defining microhabitat features. Recent and historical survey data suggest that human activities have had a significant influence on contemporary species presence/absence patterns and may have been responsible for the prominent fragmentation within its distribution.

Mitochondrial DNA (mtDNA) control region variation was used to assess genetic diversity and structure across the geographic range of *N. oxleyana*. Haplotypic diversity was highest in a small NSW subcatchment south of Evans Head (*h* = 0.594) followed by in Marcus Creek in Queensland (*h* = 0.475). Distinct genetic differentiation was evident among Queensland localities and NSW subcatchments, implying restricted gene flow between coastal river
systems. One of the nine haplotypes detected was distributed over 83.4% of the species’ range, suggesting historical connectivity among the now fragmented populations. These patterns were concordant with eustatic changes associated with the last glacial maximum. High barrier sand dunes may also act as barriers to gene flow and dispersal between adjacent NSW subcatchments. Conservation efforts should focus on the preservation of genetic diversity by maintaining as many genetically differentiated populations as possible. The relatively diverse populations inhabiting the South Evans Head subcatchment and Marcus Creek require special management consideration.

The reproductive biology of *N. oxleyana* was described from simultaneous studies of wild populations in north-eastern New South Wales and mature fish held in aquaria. In the wild, males and females matured at total lengths of 24.0-25.9 mm and 28.0-29.9 mm, respectively. In captivity, male broodfish closely guarded sites within artificial, plant-like substrate in which pairs of fish spawned adhesive eggs. Protracted serial spawning of wild and captive fish occurred from September to April/May at mean water temperatures ≥ 16.6°C and day length ≥ 10.7 hours. Captive broodfish spawned on an average of 57% of days during the 256 day spawning period. Mean relative and batch fecundities of captive females were 587 eggs/g of body weight and 7.8 eggs/fish/day, respectively. Batch fecundity of wild females was estimated at 7.8 eggs/fish. The protracted serial spawning strategy of the species may reflect an evolutionary adaptation for survival in the harsh, variable environments in which it occurs.

The developmental ontogeny and morphology of the eggs, larvae and early juveniles were described based on collections of preserved wild fish, and preserved and live captive specimens reared at 25±1°C. Eggs are telolecithal, spherical, average 1.02±0.004 mm in diameter, have a smooth chorion without filaments that adheres to spawning substrate, and follow the general pattern of teleost embryogenesis. Early, middle and late stages of embryonic development were completed on average at 16, 28 and 50 hours post fertilisation. The larvae have generalised perciform morphological development with no apparent larval specialisations. Live newly hatched larvae measured 2.8-3.4 mm in body length and commenced exogenous feeding at five days post hatch. Squamation occurred in larvae from 7.5-10.3 mm (preserved body length) with its completion determining the end of the larval stage. Given that *N. oxleyana* utilise aquatic vegetation throughout its entire life cycle, the conservation and recovery of the species depends largely on the maintenance of this habitat within remaining water bodies and associated wallum ecosystems.
The results of this study have important implications for the conservation of *N. oxleyana* in Australia. It is evident that the recovery of this species is dependent upon the protection of the particular macro-, meso- and microhabitat features shown to be associated with healthy extant populations. This will require management strategies focused on maintaining environmental drivers such as natural climatic and river flow regimes, riparian vegetation and nutrient dynamics in these dystrophic ecosystems. In addition, it will be necessary to understand the processes that govern the dispersal of this small fish along connectivity pathways in floodplain rivers, as well as identifying the main breeding populations, sources of colonists and possible movement pathways into individual drainages and isolated water bodies. In this regard, population genetics research using sensitive molecular techniques such as microsatellite markers may elucidate patterns of contemporary gene flow and dispersal among drainages as well as inform efforts to increase within-population genetic diversity of remnant populations suffering the effects of small population size. This thesis has laid the foundation for these research initiatives.
ACKNOWLEDGEMENTS

Many thanks to Prof. P. Baverstock, Dr B. Pease and Dr D. Pollard for their supervision and support. The unwavering support provided by Dr Bob Creese and Bill Talbot is also greatly appreciated. This study received financial assistance from the Australian Research Council, the NSW Department of Primary Industries (DPI), Southern Cross University, the NSW Fisheries Scientific Committee and Consolidated Rutile Ltd.

I am grateful to all of the NSW DPI scientists who provided useful discussions, comments and advice during the life of this project, including Dr M. Broadhurst, G. Butler, Dr S. Fielder, Dr D. Gilligan, Dr T. Glasby, Dr A. Jordan, Dr N. Otway, Dr S. Rowland, and Dr M. Storrie. Likewise, thank you to those who assisted with fieldwork, data collection and animal husbandry including C. Gallen, T. Fowler, P. Gibson, G. Holder, G. Housefield, R. Laird, B. McCartin, N. Reed, and I. Theibauld.

I thank all of the manuscript co-authors for their valuable contribution and considerable effort to meet important deadlines including Prof. A. Arthington, Prof. P. Baverstock, G. Butler, Dr L. Brooks, M. Elphinstone, Dr T. Glasby, C. Nock, P. Smith, Dr T. Trnski, and Dr R. Wager. Dr L. Brooks also provided exceptional statistical advice, and Dr P. Pankhurst helped make readable the ‘purple smudge’ that is a histological sample. Thanks to the staff at the Wollongbar Agricultural Institute for preparing the ‘smudges’. For comments on specific chapters I also thank Prof. A. Arthington, Dr S. Balcombe, Prof. P. Baverstock, Dr M. Broadhurst, Dr B. Creese, Dr D. Gilligan, Prof. J. Hughes, Dr D. Jerry, Dr A. Jordan, Dr J. Leis, Dr B. Pease, Dr D. Pollard, and Dr S. Rowland. The contribution made by the anonymous referees and editors of the journals where each manuscript has been or is being published is also acknowledged.

A special thank you to my good mate, Gavin Butler for simply sharing the PhD ordeal, being ready to give up his limited time to lend a hand and always being keen to discuss research at length over the phone. Similarly, thanks to the mapping magician Greg West for his friendship and help with GIS over the years.

Finally, thank you to my beautiful wife Gab for her unwavering support, love and devotion, and ability to motivate and keep me going through the tough times.
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1: General Introduction

The Decline of Australia’s Native Fish Fauna

Australia has been described as the driest inhabited continent with the lowest average rainfall and the highest proportional loss of rainfall by evaporation and transpiration (Lake et al. 1986). Despite this, the freshwater fish fauna is considered to be remarkably rich given the arid climate prevailing over much of the continent and the resultant scarcity of freshwater habitats (Gehrke 1997). In total, approximately 200 native species have been described (Merrick and Schmida 1984; McDowall 1996a; Allen et al. 2002).

Many of Australia’s freshwater fish species are under threat with their distributions and abundances having been reduced significantly since European settlement (Wager and Jackson 1993). This includes both the larger species targeted by recreational fisheries and the smaller species of high conservation value that occupy important niches within aquatic communities (Schiller et al. 1997). Approximately 8% of Australia’s freshwater fish are threatened with extinction and a further 25% are believed to have either declined significantly in distribution or have very restricted distributions. One species, the Pedder galaxias Galaxias pedderensis, is considered to be extinct in the wild (Wager and Jackson 1993; DEH 2005).

The causes of the decline of native freshwater fishes in Australia have been reviewed by many authors including Lake (1971), Cadwallader (1978), Pollard et al. (1980, 1990), Ingram et al. (1990), Koehn and O’Connor (1990), Faragher and Harris (1993), Wager and Jackson (1993), Schiller et al. (1997), Morris et al. (2001) and Pusey et al. (2004). Pusey et al. (2004) classified the processes threatening Australian fishes into seven broad categories including hydrological alteration, loss of longitudinal and lateral connectivity, changes in habitat structure, quality and chemical composition, interactions with introduced alien (i.e. introduced from overseas) and translocated native species, overexploitation, global climate change, and inadequate knowledge and understanding. Threatening processes are often interlinked, their effects can be synergistic and cumulative, and rarely is one factor alone responsible for a species’ decline (Groom 2006). For example, the effects of harvesting fish populations may be amplified in populations already under pressure from habitat degradation.

Conserving threatened species and mitigating threatening processes is becoming an increasingly important issue in today’s society and a priority issue for all levels of government. Within Australia, Commonwealth and State legislation provides for the
protection, conservation and recovery of threatened species, populations and ecological communities. Under the New South Wales (NSW) *Fisheries Management Act 1994*, statutory recovery plans aim to return a threatened species, population or ecological community to a position of viability in nature, and outline the actions that government agencies and other organisations have agreed upon to promote the recovery of the species. Recovery planning involves utilising knowledge of the biology and ecology of the threatened species to mitigate human impacts. Effective management requires a strong understanding of a species’ evolution, distribution, biology and ecology, and the environmental factors required to maintain fish populations and genetic diversity (Burgman and Lindenmayer 1998; Meffe *et al.* 2006). Unfortunately, this information is often lacking for many of Australia’s threatened fishes (Wager and Jackson 1993; Morris *et al.* 2001). Hence, research that generates relevant and strategically-important biological and ecological information forms the basis of the development and implementation of effective recovery programs.

The Endangered Oxleyan pygmy perch *Nannoperca oxleyana* Whitley

Pygmy perch are a group of small, freshwater fishes endemic to southern Australia and are recognised as some of Australia’s most threatened fishes. The three genera of pygmy perch *Edelia, Nannatherina* and *Nannoperca* are considered members of the family Percichthyidae (previously Nannopercidae) (Hoese *et al.* 2006). Recent phylogenetic analysis supports the placement of the two species of *Edelia* (*E. vittata* and *E. obscura*) within *Nannoperca* (Jerry *et al.* 2001). The Oxleyan pygmy perch *Nannoperca oxleyana* Whitley inhabits coastal mid-eastern Australia, and represents the northeastern distribution limit of these genera. The Balston’s pygmy perch *Nannatherina balstoni* Regan and western pygmy perch *Nannoperca vittata* (Castelnau) are restricted to southwestern Australia, while the Ewen pygmy perch *Nannoperca variegata* Kuiter and Allen, Yarra pygmy perch *Nannoperca obscura* (Klunzinger) and southern pygmy perch *Nannoperca australis* Günther are endemic to the southeastern corner of the country (Kuiter and Allen 1986; Kuiter *et al.* 1996). Cryptic speciation exists with *N. vittata* and *N. australis* each apparently comprised of two distinct, yet undescribed species (Hammer 2002; M. Hammer, University of Adelaide, South Australia, pers. comm.). Many populations of pygmy perch have been lost or are under threat from habitat degradation and negative interactions with introduced species. As a result, four of the six described species of pygmy perch are threatened with extinction.
*Nannoperca oxleyana* is recognised as the most threatened pygmy perch. This species grows to approximately 60 mm in total length (Knight 2000) and is characterised by a moderately compressed body, one deeply notched dorsal fin and a truncated tail (see Figure 1 in Chapter 2). Dorsal fin counts include six to eight spines and seven to nine rays. Anal fin counts include three spines and seven to nine rays (Whitley 1940; Kuiter and Allen 1986). Further distinguishing features include the absence of a lateral line, a small mouth reaching to just below the eye and enlarged teeth in its lower jaw. The body is covered in ctenoid scales and is light brown to olive in colour, darker on the back, with a conspicuous round black spot with an orange margin at the base of the caudal fin. While the fins are normally clear, during breeding the dorsal, pelvic and anal fins darken and the tail and lateral stripes turn red (Wager 1992).

*Nannoperca oxleyana* has a very restricted and patchy geographic distribution within the coastal, freshwater wallum (*Banksia* dominated heath) ecosystems of northern NSW and southern Queensland (Arthington 1996; Knight 2000; Pusey et al. 2004). Habitat destruction, fragmentation and degradation associated with intensive coastal development in this region over the last 50 to 100 years are thought to have caused a severe decline in the distribution and abundance of *N. oxleyana*, as have adverse interactions with the alien pest fish species eastern gambusia *Gambusia holbrooki* (Girard), and over-collection for aquarium purposes (Arthington 1996; Kuiter et al. 1996; NSW DPI 2005). As a consequence, *N. oxleyana* is listed as endangered by the IUCN (IUCN 2004), by the Australian Society for Fish Biology and under the Australian Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* and New South Wales (NSW) *Fisheries Management Act 1994*. The species is also listed as vulnerable under the Queensland *Nature Conservation Act 1992*. The endangered classification, although differing slightly among the various conservation listings and legislation, generally includes those taxa which have suffered a population decline over all or most of their range and are in danger of extinction in the near future unless the factors threatening their survival cease to operate (Crook 1999; NSW Fisheries 1999; Commonwealth of Australia 2000; IUCN 2000). A national recovery plan has been developed by the NSW Department of Primary Industries (DPI; formerly NSW Fisheries) to promote the recovery of *N. oxleyana* (NSW DPI 2005).
Recovery Research for *Nannoperca oxleyana*

Aspects of the conservation biology of *N. oxleyana* have been reviewed by Wager and Jackson (1993), Arthington (1996), Kuiter *et al.* (1996), Knight (2000, 2002), Thompson *et al.* (2000), Morris *et al.* (2001), Pusey *et al.* (2004) and NSW DPI (2005). Based on research priorities identified by Knight (2002), the national recovery plan outlines a number of ‘Research and Investigation Objectives and Activities’ designed to fill biological knowledge gaps and gather information necessary to plan recovery actions (Table 1). Information is required on the species’ distribution and population genetics in NSW, life history, population dynamics, ecology, and the threats posed by introduced species. The rationale for the research objectives and activities outlined in the recovery plan is discussed below.

**Research Objectives 1 and 2: Establish and map the distribution and habitats of N. oxleyana**

The most serious threat to *N. oxleyana* is habitat degradation, fragmentation and loss associated with residential development, road construction, agriculture, forestry, sand mining, drainage modification, water pollution and tourism activities (Pusey *et al.* 2004; NSW DPI 2005). Management and mitigation of these impacts requires detailed knowledge of the locations and habitat requirements of extant populations. The NSW *Fisheries Management Act 1994* contains provisions to formally protect habitat considered critical to the survival of a threatened species, population or ecological community (termed ‘Critical Habitat’). The Act also integrates the conservation of threatened species into development control processes established by the NSW *Environmental Planning and Assessment Act 1979*. As part of the development assessment process, consent authorities are required to assess development impacts on threatened species, and to consider if activities are a class of development recognised as a threatening process (NSW DPI 2005). Additionally, activities and developments that do not require approval under the *Environmental Planning and Assessment Act 1979* may require licensing under the *Fisheries Management Act 1994* if they are likely to harm a threatened species, population or endangered ecological community, or their habitat. Careful consideration must be given to the effects of proposed developments or activities on threatened species and their habitats. In this regard, the NSW DPI is responsible for providing relevant planning authorities with detailed information and maps on the precise locations of known and potential habitats occupied by a threatened species.
Table 1. Research and Investigation Activities outlined in the *Nannoperca oxleyana* recovery plan (Source: Knight 2002; NSW DPI 2005).

<table>
<thead>
<tr>
<th>Objective</th>
<th>Activity</th>
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| 1. Undertake a survey program to better establish the distribution of *N. oxleyana* and it’s habitat requirements | • Evaluate sampling methodologies to determine the most effective way to sample *N. oxleyana* populations while minimising adverse impacts on the species.  
• Conduct broad-scale surveys to establish the species’ limits of distribution and to identify catchments where the species might be found.  
• Conduct intensive surveys in drainage areas identified as supporting or potentially supporting new *N. oxleyana* populations, to map their distribution and identify habitat associations. |
| 2. Model and map known and potential *N. oxleyana* habitat | • Develop a GIS-based map of the distribution of known and predicted *N. oxleyana* habitat across the species range. This will be achieved by combining available mapping and remote sensing data (including soil and vegetation layers, drainage patterns, land tenure, zoning and other relevant planning information) with existing or new field data.  
• Produce fine scale maps for important areas (e.g. near towns) showing the distribution of water bodies: (a) known, (b) with the potential, and (c) unlikely to support *N. oxleyana*. |
| 3. Conduct genetic research to establish the degree of isolation between populations and factors influencing dispersal of the *N. oxleyana* | • Initiate a project to examine genetic diversity and structure in populations of *N. oxleyana*, in collaboration with a university or other research institution.  
• Support this work by collecting genetic material (e.g. fin clips) during surveys within NSW. |
| 4. Support research into the environmental tolerances, population dynamics and other aspects of the life history and ecology of *N. oxleyana* | • Encourage scientific investigation of key areas of the biology and ecology of *N. oxleyana* to provide information valuable to the recovery of the species or its management. This may include work to establish environmental tolerances, ability to survive in disturbed habitats, factors influencing population dynamics and variability, age and growth, diet, etc. |
| 5. Monitor populations of gambusia and other exotic or native introduced fish species within or near water bodies occupied by *N. oxleyana*, and implement measures to reduce their impacts | • Record data on all fish species captured during the survey program and on-going monitoring program for entry into a species database. The data will record the capture of introduced and indigenous species.  
• Use this data, and any other available records, to map the distribution of introduced species relative to *N. oxleyana* and any expansion in their range or abundance over time.  
• Implement the NSW Freshwater Fish Stocking Fishery Management Strategy and Qld DPIF translocation policy to prevent significant impacts from stocking on *N. oxleyana* populations. |
| 6. Study interactions between gambusia and *N. oxleyana* to better establish the degree of threat posed by gambusia | • Support further studies on interactions between the two species (e.g. experimental trials, behavioural studies, resource partitioning studies) to determine the probable impacts of gambusia on *N. oxleyana*. |
Numerous limnological and aquatic faunal surveys have documented the fish communities of the coastal heathlands and surrounding catchments of south-eastern Queensland (Arthington 1996). Despite extensive and intensive sampling, *N. oxleyana* has only been recorded from 33 localities since 1990 (A. Arthington, Griffith University, Queensland, pers. comm.). Populations are restricted to the Mary, Noosa, and Maroochy basins, and Fraser, Moreton and North Stradbroke Island (Figure 1). One record also exists for the Pine River Basin (NSW DPI 2005) but this is thought to have been a translocation into a farm dam which now no longer exists (R. Wager, Rocksberg, Queensland, pers. comm.).

In contrast, baseline data on the fish communities within the coastal heathlands of northern NSW are deficient (Knight 2000). Prior to the commencement of the recovery planning process in 2000, many of the wallum drainage systems were unsurveyed and there had been limited targeted sampling for *N. oxleyana*. Historically, the species was documented from an unspecified water body near Tatham on the Richmond River in 1929 (Whitley 1940; Figure 1). In the 1970s, the species was collected from Lake Hiawatha, Bookram Creek (called ‘Wooli Creek’ in the Australian Museum records) and Tick Gate Swamp near the township of Wooli in the Clarence River catchment, and from Cassons Creek near Red Rock in the Bellinger River catchment (Timms 1982; Llewellyn 1983; G. Schmida, Lower Beechmont, Queensland, pers. comm.). More recent locality records also come from the Wooli areas, including from Lake Minnie Water in 1995 (Lawrence 1998) and from two swamps surrounding Lake Hiawatha in 2000 (A. Lo, ANGFA NSW, pers. comm.). Three records from the Richmond River catchment also exist near Evans Head, derived from surveys during the 1990s (Arthington 1996; Walker and Walker 1999; WBM Oceanics Australia 2000). Further sampling undertaken as part of the recovery planning process was concentrated in the wallum habitats in and around Broadwater National Park and resulted in the capture of *N. oxleyana* from 24 previously undocumented localities (Knight 2000). In May 2001, three more water bodies in this area were found to support the species (Knight 2001, unpublished data).

With the exception of the Tatham area and Cassons Creek, all records occur within a large tract of coastal wallum ecosystems within or adjacent to Broadwater, Bundjalung and Yuraygir National Park. Of these, Broadwater National Park and the surrounding heathland is the only area thoroughly surveyed, accounting for 28 (85%) of recent sightings. The well-established presence of *N. oxleyana* within and adjacent to this reserve makes the area one the most important sites for the species’ long-term conservation. However, large tracts of similar habitat in NSW are yet to be thoroughly surveyed and it is plausible that the species’ known distribution may be expanded upon further investigation (Knight 2000).
Figure 1. Known distribution of *Nannoperca oxleyana* prior to May 2001. NSW localities are differentiated by year of discovery. Data sourced from the NSW DPI Aqua-See Database and A. Arthington, Griffith University, Queensland.
Knight (2002) and NSW DPI (2005) also identified a need to develop a standardised, non-destructive sampling protocol for *N. oxleyana* (Table 1). In recent years, there has been an increase in the number of studies by government organisations, universities, consultants and native fish enthusiasts aimed at documenting the location and relative size of remaining populations. As *N. oxleyana* inhabits a coastal area under increasing development pressure (Zann 1996), a standardised sampling protocol that effectively detects abundances and distributions is required. Concomitantly, there is also a need to avoid excessive sampling by establishing efficient and cost effective protocols (Angermeiser and Smogor 1995; Growns *et al.* 1996) that utilise non-destructive techniques to target populations (Kelsch and Shields 1996; Neilson 1998; Craig 2006). The application of a standardised sampling protocol would assist in reducing the risk of misreporting presence and absence patterns and ensure that data are comparable among surveys conducted over large spatial and temporal scales.

**Research Objective 3: Undertake population genetics research**

The conservation and recovery of *N. oxleyana* requires the maintenance of the existing genetic diversity and gene flow among and within extant populations. Genetic diversity influences the amount of evolutionary change within a population, gives a population adaptive potential to cope with competitors, predators, parasites, new diseases and environmental change, and affects the reproductive fitness of individuals (Burgman and Lindenmayer 1998; Scribner *et al.* 2006). A reduction in genetic diversity and changes in the distribution of this diversity among populations via population declines and fragmentation or unnatural gene flow (e.g. inappropriate restocking) can negatively affect these attributes, thereby increasing the likelihood of population and species extinction (Frankham 2005; Scribner *et al.* 2006).

Conservation population genetics aims to quantify the amount of genetic variation within and between populations, thereby providing a measure of the degree of isolation between populations (Frankham *et al.* 2002). Such information may provide insights into factors influencing the dispersal of the species, the degree of threat posed by habitat fragmentation, barriers to fish passage, and changes to topography or hydrology, assist in identifying appropriate management units and areas of importance in maintaining gene flow, prioritise actions towards conserving unique, source and declining populations, and determine the need for and appropriateness of restocking programs (NSW DPI 2005).

Genetic research by Hughes *et al.* (1999) used allozyme and mitochondrial DNA to examine the genetic structure of 215 individuals taken from nine sites throughout the species’ range in
south-eastern Queensland. Hughes et al. (1999) concluded that a combination of restricted environmental requirements and geomorphologic changes over time led to extremely limited dispersal among populations, resulting in most populations being isolated from one another for a considerable period of time. These populations are therefore likely to be vulnerable to localised catastrophic events, with re-colonisation unlikely to occur (Hughes et al. 1999).

There was however evidence of recent, historical gene flow among some of the mainland populations and of contemporary gene flow among a variety of interconnected swampy seepages and lakes on Moreton Island. The study also found that although there was little dispersal between populations in separate drainage systems, fish may move voluntarily, or be transported by high flows over quite large distances within individual drainage systems (e.g. Noosa River). These results highlight the importance of maintaining gene flow among and within populations and the importance of conserving as many populations as possible in order to preserve the species’ full genetic integrity. The results also highlight that it would be inappropriate to increase within-population genetic diversity by translocating small numbers of individuals between populations (Hughes et al. 1999).

As the distribution of *N. oxleyana* in NSW prior to 2000 was poorly known (see distribution section above), Hughes et al. (1999) sampled only Queensland populations of the species. Consequently, the degree of genetic differentiation, and thus dispersal patterns, among NSW populations of *N. oxleyana* is currently unknown. Knight (2000) suggested that dispersal of *N. oxleyana* among water bodies near Evans Head may readily occur due to the particular topography and hydrological characteristics of the area. The populations inhabited an extensive network of wallum water bodies situated on a low-lying coastal plain, which were intermittently inundated by heavy rainfalls or large floods. Intermittent connection among water bodies could potentially facilitate the dispersal of *N. oxleyana*, thereby allowing colonisation of new drainage systems or recolonisation of areas previously occupied by the species. Genetic analysis of NSW populations, replicating the methodology of Hughes et al. (1999), may test the flood dispersal hypothesis and also provide insights into historical dispersal and biogeographic patterns, as undertaken in Queensland. The research would also assist in developing and prioritising management actions for NSW populations based on a sound understanding of population structure and the most appropriate foci for the conservation of genetically differentiated populations.
Research Objective 4: Undertake biological and ecological research

To date, only limited information exists on the biology of *N. oxleyana* (Table 2). Information from field studies includes size at maturity, the seasonality of spawning and recruitment, seasonal variation in population abundance, growth rate, condition, diet, and interactions with other endemic fish species. Other attributes, such as age at maturity, courtship, spawning behaviour and growth, have been documented in aquaria (Leggett 1990; Wager 1992; Arthington and Marshall 1993; Arthington 1996). However, effective recovery actions should be based on a detailed understanding of the biology and population characteristics of the species including absolute population size; birth, growth and mortality rates; longevity; age/size distribution; age/size at maturity; sex ratio; reproductive effort; early development; larval requirements and dispersal; and recruitment processes. Concomitantly, information on environmental influences on these parameters would provide important insights into the dynamics of populations and identify environmental variables critical to the survival of the species. For example, current theory suggests that *N. oxleyana* may undergo large population fluctuations driven by the extent and timing of rainfall events, and hence creek and river discharge and lake water levels (Arthington 1996; Knight 2000). Similarly, there is a need to examine the annual reproductive cycle of *N. oxleyana* within the southern area of its distributional range, as Arthington and Marshall (1993) found evidence that, near its northern distributional limits, the species had a spawning period extending into late autumn, presumably due to prolonged high water temperatures.

*Nannoperca oxleyana* is primarily found in lotic and lentic habitats within coastal wallum water bodies that are distinguished by their low nutrient status, low salinity and low magnesium and calcium hardness (Pusey *et al.* 2004). The species’ ability to occupy a broader niche and hence survive and maintain viable populations within other habitats is unknown. There is also a general lack of information regarding the tolerance of *N. oxleyana* to the impacts of habitat degradation such as vegetation clearing, reduced water quality and prescribed burning activities. However, patterns of decline in distribution and abundance of *N. oxleyana* within degraded creeks suggest that the species may be intolerant to degraded environments (Arthington 1996).
Table 2. Life history and ecological characteristics of *Nannoperca oxleyana* derived from studies of wild and captive populations (adapted from Pusey *et al.* 2004). * indicates additional information sourced from NSW DPI (2005). TL = total length. SL = standard length. Qld = Queensland.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Information</th>
</tr>
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<tbody>
<tr>
<td>Age at maturity</td>
<td>In aquaria: 4-5 months</td>
</tr>
<tr>
<td>Minimum length of gravid females</td>
<td>30 mm TL in aquaria and Noosa R., Qld; 19 mm SL in Spitfire Ck, Qld</td>
</tr>
<tr>
<td>Minimum length of ripe males</td>
<td>27 mm TL in aquaria and Noosa R., Qld; 19 mm SL in Spitfire Ck, Qld</td>
</tr>
<tr>
<td>Longevity</td>
<td>Unknown, possibly up to 5 years</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>Unknown</td>
</tr>
<tr>
<td>Occurrence of ripe fish</td>
<td>In wild: September/October - May</td>
</tr>
<tr>
<td>Peak spawning activity</td>
<td>In wild: October - December</td>
</tr>
<tr>
<td>Critical temperature for spawning</td>
<td>In aquaria: &gt;20° C</td>
</tr>
<tr>
<td>Inducement to spawning</td>
<td>In aquaria: water temperature &gt;20° C</td>
</tr>
<tr>
<td>Mean gonadosomatic index of ripe fish</td>
<td>In wild: females: 3.3 ± 0.002%; males: 0.6 ± 0.197%</td>
</tr>
<tr>
<td>Fecundity</td>
<td>In wild: 23-270 eggs/fish</td>
</tr>
<tr>
<td>Fecundity/length relationship</td>
<td>Unknown</td>
</tr>
<tr>
<td>Egg diameter</td>
<td>Unknown</td>
</tr>
<tr>
<td>Frequency of spawning</td>
<td>In aquaria: a few eggs laid daily during a protracted spawning season</td>
</tr>
<tr>
<td>Oviposition and spawning site</td>
<td>In aquaria: demersal, adhesive eggs attaching to aquatic vegetation</td>
</tr>
<tr>
<td>Spawning migration</td>
<td>In wild: none observed</td>
</tr>
<tr>
<td>Parental care</td>
<td>In aquaria: none observed</td>
</tr>
<tr>
<td>Time to hatching</td>
<td>In aquaria: 1-3 days, 3-4 days</td>
</tr>
<tr>
<td>Length at hatching</td>
<td>Unknown</td>
</tr>
<tr>
<td>Length/age at free swimming stage</td>
<td>Unknown</td>
</tr>
<tr>
<td>Length/age at loss of yolk sac</td>
<td>Unknown</td>
</tr>
<tr>
<td>Length at first feeding</td>
<td>Unknown</td>
</tr>
<tr>
<td>Age at first feeding</td>
<td>In aquaria: 1-2 days post hatching</td>
</tr>
<tr>
<td>Length/age at metamorphosis into juveniles</td>
<td>In wild: length increased from 14 mm to &gt; 28 mm over a one year period</td>
</tr>
<tr>
<td>Growth rate*</td>
<td>Microphagic carnivore consuming prey &lt; 5-6 mm including zooplankton, aquatic insects, atyid shrimps, terrestrial arthropods, and flying aquatic insects</td>
</tr>
<tr>
<td>Trophic guild and dietary composition</td>
<td>Firetailed gudgeon (<em>Hypseleotris galii</em>), empire gudgeon (<em>Hypseleotris compressa</em>), striped gudgeon (<em>Gobiomorphus australis</em>), softspined rainbowfish (<em>Rhadinocentrus ornatus</em>), Duboulay’s rainbowfish (<em>Melanotaenia duboulayi</em>), honey blue-eye (<em>Pseudomugil mellis</em>)</td>
</tr>
<tr>
<td>Common co-occurring native fish</td>
<td>In wild: none observed</td>
</tr>
<tr>
<td>Negative interactions with native fish</td>
<td>In wild: none observed</td>
</tr>
</tbody>
</table>
Research into the physiological tolerances of *N. oxleyana* to various water quality parameters and pollutants, coupled with comparisons of distribution and abundance patterns between undisturbed and disturbed environments and along environmental gradients may assist in strengthening current knowledge regarding species-habitat associations. It may also assist in identifying environmental indicators for the species’ presence, determining the effects of habitat degradation on populations, and provide insights into factors influencing the species’ localised and large-scale distribution. This information would assist in managing remaining habitats, assessing the potential impacts of proposed developments, and evaluating the appropriateness of implementing a conservation stocking program.

**Research Objective 5: Monitor populations of introduced species**

The introduction of fish species into areas outside of their natural range is regarded as a major threat to aquatic ecosystems throughout the world (Sala *et al.* 2000) and is included under the NSW *Fisheries Management Act 1994* as a key threatening process to native fish populations. The presence of introduced species represents a major change in the local aquatic faunal assemblage and can negatively affect native fish populations through a range of impacts. These impacts include hybridisation, habitat disruption, competition for space and food, predation, and the introduction of exotic parasites and diseases (Courtenay and Stauffer 1984; Arthington and McKenzie 1997).

Throughout Australia, the distribution and abundance of introduced fish has increased dramatically in recent years, and new species are constantly being detected (Koehn and MacKenzie 2004). It is therefore imperative that any introductions of fish into environments inhabited by *N. oxleyana* be reported and monitored.

**Research Objective 6: Study interactions with eastern gambusia**

The eastern gambusia *Gambusia holbrooki* is the only alien species known to have established self-sustaining populations in the freshwater wallum ecosystems of mid-eastern Australia (Wager and Jackson 1993; Arthington 1996). This species is identified as a major threat to *N. oxleyana*. However, the suggested ecological impacts of this species in Australia tend to be anecdotal or speculative (i.e. inferred from patterns of distribution and abundance) primarily due to a lack of information on native freshwater fishes prior to its introduction (Arthington and Lloyd 1989). Similarities between the two species in terms of habitat requirements, including a preference for vegetated areas with little or no flow, coupled with the fact that both species are microphagic carnivores, implies that large populations of *G. holbrooki* could
out-compete *N. oxleyana* for essential resources (Lloyd *et al*., 1986; Arthington and Marshall 1993, 1999; Harris 1995; Arthington 1996; McDowall 1996b). *Gambusia holbrooki*’s aggressive behaviour may also force *N. oxleyana* out of optimal habitats, or impair reproductive success, growth, resistance to infectious diseases, and survival under crowded conditions during times of drought (Myers 1966; Lloyd 1990; Howe *et al*., 1997; Gill *et al*., 1999; Knight 1999; NPWS 2003). *Gambusia holbrooki* may also prey on the eggs, larvae and juveniles of *N. oxleyana*.

Research into the habitat utilisation and dietary requirements of co-occurring populations may provide insights into the level of threat that *G. holbrooki* poses to *N. oxleyana*. In addition, experimental laboratory research into the level of predation by *G. holbrooki* on early developmental stages of *N. oxleyana* and interference competition between these two species may help to quantify negative interactions inferred from the field studies. Comparative studies of the food web structure and dynamics of water bodies supporting and not supporting populations of *G. holbrooki* may also prove useful in determining the effects that this species may have on aquatic ecosystems.

**Study Aim and Objectives**

The underlying theme of this research thesis is to assist in filling the knowledge gaps outlined in the recovery plan for *N. oxleyana*. The research examines several fundamental biological attributes of the species and the ways that environmental variables and habitat destruction, fragmentation and degradation have influenced the current distribution and abundance of the species in NSW. The project contributes to an understanding of how a range of natural and human-induced influences determines the distribution and abundance patterns of threatened fish species, and aquatic fauna in general. A component of the research aims to determine levels of genetic diversity in *N. oxleyana* throughout their distributional range, and contribute to an understanding of how the genetic diversity of endangered fish species is influenced by their ability to disperse between fragmented and isolated habitats. There remains a paucity of similar studies for aquatic fauna, especially endangered Australian freshwater fishes.

The research aimed to study several poorly understood aspects of the biology of the endangered Oxleyan pygmy perch *Nannoperca oxleyana* that would assist in the recovery and conservation of the species. Specific objectives were to develop a standardised, non-destructive protocol that effectively and efficiently samples *N. oxleyana*, and to document the species’ distribution and habitat associations, genetic diversity and structure, reproductive biology and early development.
2: A SAMPLING PROTOCOL FOR THE ENDANGERED FRESHWATER FISH, OXLEYAN PYGMY PERCH NANNOPERCA OXLEYANA WHITLEY

PREFACE
This chapter has been published in the peer-reviewed journal Australian Zoologist.


Contribution to the preparation of this chapter:
Chapter Concept: I was responsible for the conception of the research.

Experimental Design: Dr T. Glasby and I contributed equally to the experimental design.

Data Collection: I was responsible for all field work. NSW DPI technical staff assisted with field work under my direction.

Data Analysis and Interpretation: I was responsible for data analysis and interpretation under the guidance of Dr L. Brooks and Dr T. Glasby.

Writing: I was solely responsible for the writing of this chapter.
The conservation of the Oxleyan Pygmy Perch *Nannoperca oxleyana* would benefit from the adoption of a standardised, non-destructive sampling protocol that effectively and efficiently detects the presence, and quantifies the relative abundance, of extant populations. The objectives of this study were to: (i) quantify the minimum number of traps required to obtain precise relative abundance estimates, (ii) assess the effectiveness and efficiency of various trapping regimes, and (iii) compare the relative detectability and short-term mortality rates of trapping, seine netting and backpack electrofishing. Previous survey data were utilised and augmented with field experiments. Ten traps provided relatively precise estimates of relative abundance. Unbaited and baited traps set for 30 and 60 minutes detected the species on all occasions, whereas traps set for 15 minutes did not. Positive correlations were found between set time and both overall (fish/trap) and standardised (fish/trap/minute) catch rates, although only the former relationship was significant. The addition of bait to traps did not significantly affect catch rates. Trapping, seine netting and electrofishing detection rates were 88%, 71% and 83%, respectively. Associated mortality rates were 10%, 55% and 0%, respectively. The deployment of multiple gear types increased the likelihood of detecting *N. oxleyana*. A sampling protocol is recommended that includes saturating sites with unbaited traps set for at least 30 minutes and sampling with a backpack electrofisher. Seine netting should be reserved for situations where an electrofisher is unavailable or non-deployable.

**Key words:** endangered fish, *Nannoperca oxleyana*, trap, seine, electrofishing, efficiency, effectiveness, mortality
Introduction

The Oxleyan Pygmy Perch *Nannoperca oxleyana* Whitley is a freshwater percichthyid fish (Fig. 1) distributed along a narrow belt of lowland, coastal habitat in mid-eastern Australia (Pusey et al. 2004). This small, bottom-dwelling, habitat specialist grows to approximately 60 mm in length and occupies shallow, swampy regions of dystrophic, acidic freshwater streams, lakes and swamps draining through sandy, wallum (*Banksia* dominated heath) ecosystems (Fig. 2) (Arthington 1996; Knight in press). Large expanses of this habitat have been destroyed or degraded by human activities. As a result, *N. oxleyana* is considered endangered and the remaining populations are typically fragmented and patchily distributed. A recovery plan has been prepared to guide long-term conservation and management programs (DPI 2005).

![Figure 1. Oxleyan pygmy perch Nannoperca oxleyana. Large mature female, 50 mm total length. Photo: DPI.](image)

In recent years, there has been an increase in the number of studies aimed at documenting the locations and relative sizes of remaining *N. oxleyana* populations. This information is fundamental to effective management, primarily because it assists with decisions regarding environmental impact and the need for habitat protection and provides a basis for developing and implementing future surveys to determine the effectiveness of recovery actions. In
reviewing the surveys undertaken to date, Knight (2000) noted inconsistencies in the documented spatio-temporal distribution patterns of *N. oxleyana*, which in part may be attributed to the relative effectiveness of various sampling regimes to capture the species.

**Figure 2.** Typical swampy habitat of *N. oxleyana* within a dystrophic, acidic, freshwater stream near Evans Head, New South Wales. The ‘red’ emergent macrophyte *Philydrum lanuginosum* typically dominates the littoral zone and provides important habitat for the fish. Photo: J. Knight.

The main techniques used to assess distributions have included sampling with small collapsible traps set either unbaited or baited for between 15 and 90 minutes, as well as active gear such as seine nets and backpack electrofishers (e.g. Arthington 1996; Bishop 1999; Esdaile 2000; Knight in press). Dip netting has also been used on occasion (e.g. Leggett 1990).

As with all fish sampling methods, each of the above gear types may be spatially or temporally biased towards sampling particular species of fish (Hayes *et al.* 1996; Hubert 1996). When designing a study, it is important to select sampling gear types that are the least biased and provide the most accurate data possible (Willis and Murphy 1996). The ability to accurately detect a rare species such as *N. oxleyana* may, however, be hindered by its reduced
distribution and abundance (Green and Young 1993; Jackson and Harvey 1997). Given that the conservation management of a threatened species such as *N. oxleyana* relies heavily upon robust scientific data, it is imperative that a standardised sampling protocol is adopted that effectively detects presence and absence patterns and gives reliable estimates of relative abundance.

Spatial and temporal comparisons of population size are best derived by standardised sampling methods. To date, trapping has been the primary technique used to study the ecology of *N. oxleyana* populations (Arthington and Marshall 1993; Arthington 1996; Knight 2000, in press). When examining changes in relative abundance through estimates of catch per unit effort (CPUE), an optimal level of precision is required (Kingsford 1998). Concomitantly, there is also a need to avoid excessive sampling by establishing efficient and cost-effective protocols (Angermeyer and Smogor 1995; Growns *et al.* 1996).

Sampling protocols for threatened species should aim to utilise the least invasive methods and avoid techniques that may be destructive to both the target population and its habitat (Kelsch and Shields 1996; Neilson 1998; Craig 2006). While trapping, seining and electrofishing have been used to catch *N. oxleyana* in a range of habitats, the associated fishing mortality of each gear type has not been quantified. All gear has the potential to cause short- or long-term injuries that could negatively affect health, behaviour, growth, and reproduction or ultimately cause death (Kelsch and Shields 1996; Snyder 2003). Techniques such as seine netting are also capable of disturbing fish habitats (Ivantsoff *et al.* 1988; J. Knight, personal observations).

Given the above, this study aimed to develop a standardised, non-destructive sampling protocol that effectively and efficiently targeted *N. oxleyana*. The specific objectives were to: (i) quantify the minimum number of traps required to obtain precise relative abundance estimates, (ii) assess the effectiveness and efficiency of various trapping regimes to capture *N. oxleyana*, and (iii) compare the relative detectability and short-term mortality rates of trapping, seine netting and backpack electrofishing. These analyses were restricted to *N. oxleyana* because an adequate data set on sympatric species was not available.
Methods

Gear specifications

Fish traps were similar to those used in previous studies of *N. oxleyana* and other small-bodied fishes (Arthington and Marshall 1993; Arthington 1996; Bishop 1999; Balcombe and Closs 2000; Knight 2000, in press). Traps measured 250 x 250 x 450 mm and consisted of 3 mm nylon mesh covering a rectangular, collapsible wire frame with an open, inverted funnel at each end with 40 mm openings. The seine net, which was specifically designed to sample the narrow, overgrown drainage systems typical of the wallum ecosystems, was constructed from 3 mm, knotless (i.e. non-abrasive), polyester nylon mesh, measured 4 x 1.5 m, had a centred pocket 1 m in length, a weighted foot-rope and a floating head-rope. Electrofishing was done with a Smith-Root model 12B, battery powered, backpack electrofisher, using a 280 mm diameter aluminium anode ring attached to a fibreglass handle, and a rat-tail cable cathode. Depending on conductivity, electrical output ranged from 200 to 500 volts of pulsed direct current (DC) at a fixed pulse rate of 60 Hz. Affected fish were collected using a dip net constructed with the same mesh as that used for the seine net.

Trapping regimes and precision

The minimum number of traps required to gather precise estimates of relative abundance was determined through analysis of the relationship between the number of traps and sampling precision (standard error, S.E.) (Andrew and Mapstone 1987; Kingsford 1998). CPUE data were derived from five sets of 18-40 traps set unbaited for 30 minutes in two lentic and one lotic system near Evans Head, New South Wales (NSW), Australia (29° 07’S. 153° 26’E). For each given number of traps within each of the five data sets, the S.E. of the accumulated catch was calculated. The mean S.E. and its variance (± S.E.) were computed and plotted against the number of replicate traps as a negative decay curve.

A trapping field experiment was undertaken to determine the effect of bait and set time on detection rates and relative abundance estimates of *N. oxleyana*. The experiment was run over four consecutive days from 17 to 20 August 2004 in a 1.5 hectare swamp near Evans Head. The site was selected due to its homogeneous habitat and because it was known to support a large population of *N. oxleyana* (mean CPUE of 1.1 fish/trap/0.5 hour versus a mode for NSW *N. oxleyana* sites of 0.1 fish/trap/0.5 hour; J. Knight, unpublished data). Low abundances of other fish species inhabited the swamp and were rarely caught. The study site had a uniform depth of 0.5 m and a sand substratum covered by a thick layer of detritus. The emergent macrophyte *Philydrum lanuginosum* and submerged aquatic moss *Sphagnum*...
A sampling protocol for *Nannoperca oxleyana* 

*falcatulum* dominated the littoral zone and provided the majority of fish habitat. The uniformity of habitat characteristics presumably provided a relatively uniform distribution of fish within the swamp, which would minimise variability between experimental replicates (Montgomery 2005). During the experiments, water temperatures ranged from 13 to 18°C and conductivity was approximately 132 µS/cm⁻¹.

Treatments included unbaited and baited traps set for 15, 30 and 60 minutes. Unbaited traps were set on days 1 and 3 and traps baited with a combination of one 3 g cracker biscuit and 20 g of chicken pellets were set on days 2 and 4. Within a day, three sites spread evenly throughout the swamp were sampled simultaneously on three trial occasions. Within a site, during each ‘within-day’ trial, a group of 10 traps (sample size derived from precision estimates, see results) were set at 1.5-2.0 m intervals on the substrate for a standardised set time of either 15, 30 or 60 minutes. To minimise any site variability, set time was systematically rotated among sites for each sampling trial. This systematic rotation was also included across experimental days so that sites were not repeatedly sampled with the same set time for a given replicate. At the end of the 15 and 30 minute set time treatments, the traps were collected and fish removed, identified and counted. Fish were transferred to a bucket in the shade and the emptied traps were then reset to standardise overall sampling effort within and among sites. Any additional fish captured in these traps were not included in the study. After 60 minutes, all traps were pulled, fish caught in traps set for the full 60 minutes were counted and then all fish collected during the trial were released at their place of capture.

Data from the trapping field experiment were analysed with the software program Multilevel Modelling for Windows (MLwiN 1.1; Rasbash et al. 2000) to examine the treatment effects on overall (fish per trap) and standardised (catch per trap per minute, CPUE) catch rates. A Multilevel Poisson Regression Model was chosen as it dealt specifically with hierarchical random factors, used a flexible estimation method (2nd Order Partial Quasi Likelihood approximation) and efficiently analysed the positively skewed count data by employing a log-link function for the Poisson distribution. An Extra Poisson model provided an appropriate fit of the over-dispersed data (i.e. variance > mean). Two independent models were tested for systematic variation within a hierarchical sampling structure, which included sites, days within sites, trials within days, and traps within trials. The models predicted the log probability of the dependent variables, fish/trap and CPUE, varying in relation to bait type, set time, and combinations of these predictors. Given that a Poisson model deals intrinsically with count data, estimates for the CPUE model were adjusted for per minute of set time by including an offset in the model. This is analogous to modelling CPUE data. The model
outputs included a partial coefficient and the S.E. of each predictor. The partial coefficient represents the relationship between the variances of the predictor and dependent variable not explained by the other predictors. The significance of an effect parameter, which estimated the difference between a pair of predictors, was determined by the Wald Statistic, as expressed by the equation: Wald = coefficient/S.E. The Wald Statistic was squared and compared to the Chi-squared distribution with one degree of freedom so that statistics ≥ 3.84 were two tailed significant to \( p < 0.05 \). For each pair of significant predictors, tests for significant differences were made using pairwise comparisons. \( p \)-values were adjusted for multiple comparisons using the Bonferroni procedure. Model estimated mean catch rates ± 95% joint confidence intervals were calculated for each significant predictor. The model was parameterised using treatment contrasts with the first level of a predictor being taken as the intercept. For further details, refer to Snijders and Bosker (1999).

**Gear related detection rates**
Comparisons of the relative efficacy of trapping, seine netting and backpack electrofishing to detect *N. oxleyana* were made by examining survey data collected by Knight (2000, in press, unpublished data). Species detection was defined as the percentage of sampled water bodies known to support the species in which at least one specimen was detected, with a detection success of 75% or greater considered high (Reynolds 1996). Depending on site conditions, between nine and 40 unbaited traps were set for 15 to 30 minutes at 1.5-2.0 m intervals on the substrate within a range of microhabitats including aquatic vegetation, steep or undercut banks fringed with overhanging vegetation and open waters. Backpack electrofishing and seine netting were also used within similar microhabitats. Site conditions (e.g. conductivity, depth, presence of woody debris and other obstructions) generally dictated if active techniques could be deployed and which technique was used. In sites suitable for active sampling, conditions also affected the amount of sampling effort, thereby restricting sampling to 2-3 seine shots or 10 minutes of electrofishing. Restricted sampling effort is often an inherent problem when sampling freshwater fish and has been noted by other authors (e.g. Chessman 2006).

**Gear related mortality**
A field experiment was carried out to assess the short-term *N. oxleyana* mortality rates of trapping, seining and electrofishing from 12-16 August 2004 in the same water body used for the trapping study. Sampling involved setting unbaited traps for 30 minutes, and undertaking seine net shots covering 20 m² and two minute electrofishing shots (500v, 60Hz pulsed DC).
Sampling was repeated until 20 *N. oxleyana* per technique were collected. After capture, fish were quickly examined for signs of any external injuries before being randomly transferred to one of two sealed traps per technique giving 10 fish per trap. Traps were wrapped in 70% shade cloth to aid in the protection of fish from direct sunlight and potential predators, labelled with the gear type used and submerged in the swamp. After 96 hours, the numbers of live and dead fish were recorded and the total lengths of a random subsample of 26 live and dead fish were measured to the nearest 0.1 mm. Live fish were again assessed for injuries before being released.

A Kolmogorov-Smirnov two-sample test was used to test for any significant differences between the lengths of live and dead fish.

**Results**

**Trapping regimes and precision**

The relationship between the numbers of traps and sampling precision is depicted in Fig. 3. Mean S.E. decreased rapidly from 0.70 for 2 traps to 0.37 for 5 traps, gradually reduced to 0.26 for 10 traps and declined little thereafter. Similarly, variation in mean S.E. decreased and remained relatively constant for 10 or more traps. Hence, a set of 10 traps was considered to provide relatively precise estimates of CPUE, with increases in effort beyond this point only marginally increasing precision.

![Graph showing the relationship between the number of traps and sampling precision](image)

**Figure 3.** Relationship between the number of traps and sampling precision. Bars represent the variance (± S.E.) of the mean S.E. n = 5.
A sampling protocol for *Nannoperca oxleyana*

For the trapping field experiment, the overall minimum and maximum numbers of traps set on each sampling occasion for each treatment that detected *N. oxleyana* is given in Table 1. Regardless of the baiting technique employed, a set time of 60 minutes had the highest minimum and maximum detection rates while 15 minutes had the lowest. Traps set for 30 and 60 minutes caught individuals on all sampling occasions, whereas those set for 15 minutes failed to catch any individuals on two occasions.

**Table 1.** Overall ranges for the numbers of traps set on each sampling occasion that detected *N. oxleyana*.

<table>
<thead>
<tr>
<th>Set time (min)</th>
<th>Unbaited traps</th>
<th></th>
<th>Baited traps</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>60</td>
<td>5</td>
<td>10</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

Positive correlations existed between mean fish/trap and set time for unbaited and baited traps (Fig. 4a) and between mean CPUE and set time for unbaited traps (Fig. 4c). However, mean CPUE peaked for baited traps at 30 minutes before declining slightly at 60 minutes (Fig. 4c). Fish/trap and CPUE varied little between unbaited and baited traps for the first replicate day of each treatment (days 1 and 2 of the experiment) (Fig. 4b and 4d). Similar catches were also recorded for unbaited traps on the second replicate day (day 3). However, the 2nd replicate for baited traps had a greatly reduced catch rate (day 4), possibly as a result of fish exhibiting trap shyness or bait avoidance on the final day of the experiment. Mean fish/trap and CPUE for baited traps set for 30 and 60 minutes was lower on this day, although S.E. remained similar between each bait treatment for a given set time when data were pooled (Fig. 4a and 4c). Consequently, the multilevel modelling results suggested a day effect on catch rates (Table 2), but this random effect was not significant ($\chi^2 = 3.02, p>0.05$).

The fish/trap multilevel model initially included set time, bait and the bait-set time interactive terms but no significant effects of the latter two terms on catch rate were found and so these were removed (Table 2). In addition to set time, the extra Poisson variance was also significant ($\chi^2 = 166.56, p<0.0001$), thereby indicating that the catch data were over dispersed. This clumping suggests that catches were density dependant. No significant differences were found between CPUE and any of the fixed effects. Coefficients and S.E.s for the random effects were the same as for the fish/trap model.
A sampling protocol for *Nannoperca oxleyana*

**Figure 4.** Mean (+ S.E.) fish/trap and CPUE (fish/trap/minute) for unbaited (□) and baited traps (■) for a) and c) the three set times and b) and d) replicate days. Means and S.E. reflect within day trial replication. n = 6 for a) and c), n = 3 for b) and d).

**Table 2.** Parameter estimates for the fish/trap multilevel Poisson regression analysis of trap set times.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Coefficient</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept (15 minutes)</td>
<td>-1.451</td>
<td>0.325</td>
</tr>
<tr>
<td>15 vs 30 minutes</td>
<td>0.905</td>
<td>0.266</td>
</tr>
<tr>
<td>Random effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>0.037</td>
<td>0.170</td>
</tr>
<tr>
<td>Day</td>
<td>0.497</td>
<td>0.286</td>
</tr>
<tr>
<td>Extra Poisson variance</td>
<td>1.510</td>
<td>0.117</td>
</tr>
</tbody>
</table>
For the fish/trap model, Bonferroni adjusted multiple comparisons between the three set times were all significantly different (Table 3). As expected from the raw data (Fig. 4a), the model estimated a positive correlation between fish/trap and set time (Table 4). On average, 1.28 *N. oxleyana* were expected to be caught in each trap set for 60 minutes. This estimate was 2.2 and 5.6 times larger than the mean for 30 and 15 minutes, respectively. Likewise, the model estimated mean for the 30 minute treatment was 2.5 times higher than the mean for 15 minutes. Note that the upper and lower joint confidence intervals are not symmetrical as they were derived from logged values (Table 4).

### Table 3. Significance test results of set time multiple comparisons derived from analysis of fish/trap data; $\alpha = 0.017$, Bonferroni adjusted. Wald Statistic = coefficient/S.E.

<table>
<thead>
<tr>
<th>Set time comparisons</th>
<th>Wald Statistic</th>
<th>Chi-square value</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 vs 30 minutes</td>
<td>3.402</td>
<td>11.571</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>15 vs 60 minutes</td>
<td>6.858</td>
<td>47.081</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>30 vs 60 minutes</td>
<td>4.288</td>
<td>18.289</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### Table 4. Unlogged estimated mean fish/trap catch rates and joint confidence intervals for each set time treatment.

<table>
<thead>
<tr>
<th>Set time (min)</th>
<th>Estimated mean fish/trap</th>
<th>Joint confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>- 95%</td>
</tr>
<tr>
<td>15</td>
<td>0.23</td>
<td>0.095</td>
</tr>
<tr>
<td>30</td>
<td>0.58</td>
<td>0.265</td>
</tr>
<tr>
<td>60</td>
<td>1.28</td>
<td>0.614</td>
</tr>
</tbody>
</table>

### Gear related detection rates

Knight (2000, in press, unpublished data) caught *N. oxleyana* in 55 water bodies. Traps were deployed in 50 of these localities and detected the species on 88% of sampling occasions (Table 5). Although less regularly utilised, the backpack electrofisher detected fish at a similar rate to trapping, whereas the seine only succeeded in sampling individuals at 71% of the sites in which it was hauled. In total, 38 of the 55 localities inhabited by the species were sampled with multiple gear. When deployed together, both traps and seine nets retained *N. oxleyana* on 61% of sampling occasions, while traps and electrofishing both retained fish on 50% of occasions. However on a number of occasions each technique succeeded in catching individuals while the concomitant gear did not. The majority of the 17 localities not sampled...
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with multiple gear were sampled by trapping alone, with 2 localities sampled solely by backpack electrofishing and 3 localities by dip netting.

**Table 5.** Effectiveness of sampling gear to detect *N. oxleyana* from 55 inhabited water bodies including the number of localities in which a) each gear was deployed, and b) and c) multiple gears were deployed. Detection rate is defined as the number of localities in which *N. oxleyana* was successfully sampled by a) each gear, b) both gear types deployed, and c) only one of the two gear types deployed. Data derived from surveys by Knight (2000, in press, unpublished data).

<table>
<thead>
<tr>
<th>Sampling gear comparisons</th>
<th>No. of localities gear deployed</th>
<th>Detection rate Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Single gear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traps</td>
<td>50</td>
<td>44</td>
<td>88</td>
</tr>
<tr>
<td>Seine</td>
<td>28</td>
<td>20</td>
<td>71</td>
</tr>
<tr>
<td>Electrofisher</td>
<td>12</td>
<td>10</td>
<td>83</td>
</tr>
<tr>
<td>Dip net</td>
<td>3</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>b) Multiple gear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traps and seine</td>
<td>28</td>
<td>17</td>
<td>61</td>
</tr>
<tr>
<td>Traps and electrofisher</td>
<td>10</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>c) Multiple gear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only traps successful</td>
<td>38</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Only seine successful</td>
<td>28</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Only electrofisher successful</td>
<td>10</td>
<td>3</td>
<td>30</td>
</tr>
</tbody>
</table>

**Gear related mortality**

All 20 *N. oxleyana* caught with the electrofisher and held for 96 hours survived (Fig. 5). The electrical output (500v, 60Hz pulsed DC) induced galvanotaxis (forced swimming) in *N. oxleyana* sufficient enough to allow capture without causing tetany (muscle contraction, rigidity, loss of equilibrium) (see Reynolds 1996). Upon capture and at the time of release, all fish were actively mobile with no visible signs of injury. Similar observations were made for fish caught in traps, although 10% of fish died in each replicate. The mortality rate from seine netting was highest, with a mean of 55% (S.E. ± 5.0 ) of the fish captured with this technique deceased after 96 h. After capture, most fish appeared to have external injuries including loss of mucus and scales and integument abrasions. Only 9 fish were released after 96 h, with 3 displaying external injuries and inhibited swimming ability. There was no significant difference between the total lengths of live and dead fish used in the experiment (*D* = 0.308, *p*>0.1, *n* = 13, size range = 18-42 mm).
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**Figure 5.** Mean (+ S.E.) survival of *N. oxleyana* captured by backpack electrofishing, trapping and seine netting. n = 2 replicate sealed traps per sampling technique.

**Discussion**

This study has highlighted the effectiveness of using multiple techniques when sampling to detect rare species. The deployment of multiple gear maximises the chances of detecting a species because the selectivity of one particular gear may be offset by that of another (Angermeier and Smogor 1995; Jackson and Harvey 1997). For example, the catching power of passive gear such as traps partially depends on fish behaviour, which in turn may be influenced by environmental parameters like water temperature (Stott 1970; Hubert 1996). Therefore, although *N. oxleyana* is typically prone to capture by traps, during times when fish activity is hindered by extremes in temperature, the species may be more effectively sampled by an active gear such as a backpack electrofisher.

While Knight (2000, in press, unpublished data) used multiple gear where possible, environmental conditions dictated which techniques were used and at times resulted in the deployment of only one gear type. In particular, traps were deployed in 17 sites that precluded the use of active gear. These small, lightweight, collapsible and hence highly portable traps are well suited to sampling small, bottom dwelling, cryptic fishes such as *N. oxleyana*. They are also cost effective, being relatively cheap to purchase and requiring minimal maintenance and effort to deploy (Bloom 1976; Swales 1987; Hubert 1996; Balcombe and Closs 2000). They do however, require a water depth of at least 200 mm to be effective, and are prone to some variability in catch rates. But, as discussed by other authors (e.g. Bagenal 1972; Hubert
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1996), the results presented here demonstrate that such variability can be reduced by increasing sampling effort and standardising sampling methods.

This study attempted to standardise trapping methods by investigating differences in detection and catch rates between unbaited and baited traps and among various set times. The addition of bait to a trap did not influence detection rates or significantly affect catch rates. These results support other studies comparing the catch rates of *N. oxleyana* and sympatric species in traps either unbaited or baited with several different fish attractants (Arthington and Marshall 1993; Knight 2000). Unbaited trapping is therefore recommended as the preferred sampling method for future studies, as it is a faster and therefore more efficient method than baiting traps, requires less gear maintenance and eliminates unnecessary, albeit low costs associated with purchasing bait. Baited traps also have the disadvantage that they may create biases in other research such as microhabitat selection studies, as any element of attraction created by the bait may draw fish from other habitats, rather than reflect fish abundance in the habitat being sampled (Culp and Glozier 1989; Arthington 1996; Balcombe and Closs 2000).

The efficiency of a predetermined set time to catch a species largely depends on environmental factors, fish behaviour, and population densities (Swales 1987). The positive correlation between CPUE and set time and the extra Poisson variance indicate that catch rates were density dependent. Hence, it appears that as more fish entered a trap more followed. Although *N. oxleyana* does not exhibit schooling behaviour, fish have been observed to move in pairs or in small groups (Pusey *et al.* 2004).

The significant positive relationship between fish/trap and set time and the non-significant trend in increasing CPUE over time may also be related to the high density of *N. oxleyana* in the swamp with the set times doing little to saturate the traps or deplete the numbers of fish available to enter the traps. Under these circumstances, longer set times may have been required for catch rates to begin to plateau. The density of fish is also likely to have influenced the detection rates of each set time in a similar manner (Swales 1987).

The lack of a significant increase in standardised catch rates over time suggests that 15 minutes is the most cost effective time to set traps. Of particular interest, however, is that, despite the high fish densities, the 15 minute set time failed to detect the species on two occasions. It therefore seems prudent to suggest that traps should be set for a minimum of 30 minutes to maximise the likelihood of detecting *N. oxleyana*. This set time resulted in a high detection rate in targeted surveys by Knight (in press) and located populations that were not detected in previous surveys. As evidenced by the fish/trap data, a 30 minute set time also
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provided higher overall catches than 15 minutes, which in turn would give a data set with less zeros and greater precision and hence allow for more powerful statistical analysis of spatial and temporal patterns in relative abundances.

The minimum number of 10 traps was found to obtain precise estimates of relative abundances. When undertaking monitoring studies, this may be considered the most cost-effective sampling strategy, as increases in effort beyond this point provided only marginal increases in precision. However, the number or density of traps required to detect *N. oxleyana* within a water body was not examined. Low sampling effort is more likely than greater effort to miss rare species, therefore reducing the accuracy and precision of a sampling strategy and inflating perceived variability in their distribution (Angermeier and Smogor 1995; Jackson and Harvey 1997). Given that failing to detect *N. oxleyana* may lead to inaccurate conclusions regarding its distribution, and negatively affect the conservation and recovery of the species, it is recommended that a large number of traps be deployed. The numbers set should ideally be dictated by the amount of habitat available for sampling. Obviously, practical considerations also play a role, with 40 traps found to be the maximum number that can be carried by a two-person team loaded with a backpack electrofisher, a seine net and other sampling gear when hiking into areas inaccessible by vehicles. Effects of trap density were not experimentally investigated but experience indicates that traps set approximately 1.5-2 m apart effectively sample an area without unnecessarily duplicating effort. Using this criterion in situations where available *N. oxleyana* habitat is limited, at least 10 traps usually can be deployed. Finally, Arthington (1996) recorded higher catch rates of *N. oxleyana* at 1600 hours than at 0800 hours. This information may be useful when planning monitoring programs or small distribution surveys but is difficult to apply when surveying numerous sites within limited time frames.

Although less successful than trapping, the active sampling gear used to survey for *N. oxleyana* also had a moderate to high detection rate. This is despite limited sampling effort associated with restrictive site conditions providing catch data, which may have underestimated or given low precision estimates of the relative effectiveness of the two active gear types. Site conditions also resulted in active gear being deployed in fewer water bodies than the passive gear, thereby illustrating some of constraints of these techniques. The use and effectiveness of each of the active gear types is normally dictated by field conditions and the target species. For example, electrofishing is often more suitable than seining for sampling large fish and in areas obstructed by dense aquatic vegetation or woody debris. Conversely, seining is generally more effective at sampling small fish and may be used in water bodies
that are too saline or deep to electrofish (Dauble and Gray 1980; Weaver et al. 1993; Onorato et al. 1998; J. Knight, personal observations). Dip netting is generally most effective at catching *N. oxleyana* in habitats too shallow or small to adequately sample with other techniques because the species can be quite mobile and evasive (Arthington 1996; J. Knight, personal observations). The high detection rate of fish by backpack electrofishing highlights the susceptibility of this small-bodied fish to this technique and suggests that the electrical output settings (200-500v, 60Hz pulsed DC) used were effective.

The appropriateness of the electrical output is further demonstrated by the behaviour of the target fish. These settings induced galvanotaxis in *N. oxleyana* sufficient enough to allow capture without causing tetany. An electrical waveform that is too intense will induce tetany and may cause injury or death, depending on the size and the species (Reynolds 1996). Similar affects can occur within close proximity to the anode and also depending on the type of electrical current used. As such, the aim of electrofishing is to stimulate a behavioural response in fish that will result in their capture while avoiding injury and minimising stress (Reynolds 1996). The results of this study are similar to those often reported, with mortality being generally uncommon (Snyder 2003, but see Henry et al. 2004). If electrofishing results in death, it generally occurs quickly (McMichael 1993; Reynolds 1996; Henry et al. 2004) with most minor or moderately injured fish usually surviving and appearing to behave normally (Neilson 1998).

Seine netting is often considered a relatively benign technique that kills few fish (Dauble and Gray 1980; Onorato et al. 1998; Kelsch and Shields 1996), although habitat disturbance has been reported (Onorato et al. 1998). Dauble and Gray (1980) noted that some mortality did occur as a result of physical injury when rocks were caught in the net. In a similar way, the high mortality and external injury rate recorded in the current study may be at least partially attributed to the amount of organic material collected in the seine. The net dragged macrophytes, sedges and detritus from the soft sandy substrate and accumulated large quantities of aquatic moss *Sphagnum falcatum*. This debris may have resulted in the injuries observed, including loss of mucus and scales and integument abrasions. The netting used could have contributed to these injuries, but attempts were made to minimise this affect by using knotless (i.e. non-abrasive), polyester nylon mesh. The observed external injuries may have lead to reduced fitness and/or disease and then death (Kelsch and Shields 1996).

The survival rate of fish caught in traps was high although there was a 10% mortality rate. While these fish may have had reduced fitness prior to being captured, it is likely that they sustained injuries by being unintentionally dragged across the abrasive trap mesh when
removed. To this end, care should be taken when removing fish. Handling fish with wet hands or gloves and avoiding unnecessary contact should also be considered (Kelsch and Shields 1996; NHMRC 2004). Given that the species is small in size and tends to display more cryptic and evasive behaviours than other sympatric species (e.g. eleotrids, melanotaeniids, poeciliids), traps should also be thoroughly checked as fish are regularly found buried under overlapping seams in the trap mesh or lying motionless in the corners.

It is acknowledged that there may have been additional mortalities or injuries associated with each gear that were not detected in this study. For example, unaccounted mortality could have occurred to fish that actively escaped from a gear, or that died and dropped out of the gear, prior to the catch being removed from the water (Broadhurst et al. 2006). Unaccounted delayed mortality may have also resulted as a consequence of seine netting’s degradation of the aquatic environment (ICES 2004), particularly given that aquatic vegetation forms an important part of the preferred cover, feeding, breeding and nursery habitats for *N. oxleyana* (Arthington 1996; Knight 2000, unpublished data). Similarly, it is possible that internal electrofishing injuries and associated long term effects on health, behaviour, growth and reproduction may have occurred (Reynolds 1996; Snyder 2003). Elucidation of any additional harmful effects should be the subject of future research, perhaps utilising captive reared specimens or surrogate species of similar size, morphology and behaviour.

In summary, a protocol for surveying *N. oxleyana* populations is recommended whereby sites are saturated with unbaited traps set for at least 30 minutes and concomitantly sampled with backpack electrofishing. When deriving trapping estimates of relative abundance, a total of 10 traps provides the most efficient sampling strategy. Until further information on the effects of electrofishing on *N. oxleyana* are known, the technique should be used with caution and set at the minimum output necessary to be effective. Given the high mortality rate of seine netting and its associated habitat disturbance, this technique should be reserved for situations where an electrofisher is unavailable or non-deployable. As seine netting has been shown to be reasonably effective in detecting *N. oxleyana*, the decision to use the technique should be based on weighing its potential destructiveness against the importance of accurately documenting the species’ distribution. Dip netting may supplement this sampling protocol and may be useful in areas unable to be sampled effectively with the primary techniques. Regardless of the sampling method employed, appropriate techniques for the removal, care and handling of fish should be adopted.
Acknowledgements

This paper is from PhD research undertaken by J. Knight, and supported by the NSW Department of Primary Industries, Southern Cross University, Lismore, and the Australian Research Council. We thank Natalie Reed and Graham Housefield for assisting with fieldwork. Special thanks to Matt Broadhurst and Bruce Pease for comments on drafts of the manuscript. This research followed animal care and ethics protocol 3/14 approved by Southern Cross University.

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**Knight, J.T. 2000.** Distribution, population structure and habitat preferences of the Oxleyan pygmy perch *Nannoperca oxleyana* (Whitley 1940) near Evans Head, north-eastern New South Wales. BApplSc (Hons) Thesis, Southern Cross University, Lismore, NSW.

**Knight, J.T. in press.** Distribution and conservation status of the endangered Oxleyan pygmy perch *Nannoperca oxleyana* in New South Wales. NSW Department of Primary Industries, Fisheries Final Report Series.


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3: DISTRIBUTION AND HABITAT ASSOCIATIONS OF THE ENDANGERED OXLEYAN PYGMY PERCH, *NANNOPERCA OXLEYANA* WHITLEY, IN EASTERN AUSTRALIA

**Preface**
This chapter has been published online in the peer-reviewed journal *Aquatic Conservation: Marine and Freshwater Ecosystems*.


**Contribution to the preparation of this chapter:**
**Chapter Concept:** Prof. A. Arthington and I were equally responsible for the conception of the research.

**Experimental Design:** Prof. A. Arthington and I contributed equally to the experimental design.

**Data Collection:** Prof. Arthington contributed all data on the species in Queensland and I contributed all data on the species in NSW. The distribution, mesohabitat and microhabitat data collected near Evans Head, NSW was derived from my Honours Thesis: Knight, J.T., 2000. Distribution, population structure and habitat preferences of the Oxleyan pygmy perch *Nannoperca oxleyana* (Whitley 1940) near Evans Head, north-eastern New South Wales. Unpublished Honours Thesis, School of Resource Science and Management, Southern Cross University, Lismore, NSW.

**Data Analysis and Interpretation:** Prof. A. Arthington and I contributed equally to data analysis and interpretation. Included in my contribution was a statistical re-analysis of the meso- and microhabitat data derived from my Honours Thesis.

**Writing:** Prof. A. Arthington and I contributed equally to writing this chapter.
Distribution and habitat associations of the endangered Oxleyan pygmy perch, Nannoperca oxleyana Whitley, in eastern Australia

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ABSTRACT

1. Detailed knowledge of habitat requirements is particularly relevant to the conservation of rare and threatened fish species because habitat fragmentation and loss are usually the major threats to species with limited distributions and restricted habitat requirements, and habitat restoration is typically the first step in species’ recovery plans. This paper documents the macro-, meso- and microhabitat habitat associations of a small threatened Percichthyid, the Oxleyan pygmy perch, \textit{Nannoperca oxleyana}, in south-eastern Queensland and north-eastern New South Wales (NSW), Australia.

2. The species’ range encompasses approximately 530 km of coastline from Coongul Creek on Fraser Island, Queensland (25° 16’ S, 153° 09’ E) south to Tick Gate Swamp near the township of Wooli, NSW (29° 54’ S, 153° 15’ E). It is confined primarily to dystrophic, acidic, freshwater systems draining through sandy coastal lowlands and \textit{Banksia}-dominated heath ecosystems.

3. Both lentic and lotic environments provide habitat for \textit{N. oxleyana} but the species is found only in slow flowing pools and backwaters of river channels and tributaries as well as in swampy drainages, lakes, ponds and dams.

4. Trapping studies found that an abundance of structural aquatic habitat was a defining microhabitat feature either in the form of beds of emergent or submerged plants or the presence of steep/undercut banks fringed with the semi-submerged branches and fine rootlets of riparian vegetation. When present, leaf litter and snags also provided cover.

5. Recent and historical survey data suggest that human activities have had a significant influence on contemporary species presence/absence patterns and may have been responsible for the prominent gaps within the Queensland-NSW distribution of \textit{N. oxleyana}.

6. The distinctive relationships of \textit{N. oxleyana} with features of aquatic habitat at the macro-, meso- and microhabitat scale demonstrate principles applicable to any study focused on the conservation of an endangered fish species.
INTRODUCTION

Ecological processes influencing the distribution and abundance of aquatic species vary across scales of space and time. This variability presents significant challenges for ecologists seeking to determine the habitat requirements of freshwater fish in riverine environments, where there can be considerable natural spatial and temporal variability in habitat structure within and among streams, rivers and inter-connected wetlands (Frissell et al., 1986; Hawkins et al., 1993; Pusey et al., 1993). The idea that streams and rivers can be viewed as hierarchical systems, with microhabitats defined as patches of relatively homogeneous physico-chemical features occurring within larger mesohabitat units such as pools and riffles originated with the seminal work of Frissell et al. (1986). Mesohabitats comprise a stream reach, contained within a river segment, which in turn forms part of larger macrohabitats including the catchment of a single tributary, and large river basins made up of such tributaries (Frissell et al., 1986). Lentic systems such as lakes are considered segment-level units of a stream system and, while lacking river flow and hence lotic mesohabitats, may contain an array of microhabitats (Frissell et al., 1986).

Understanding the macro-, meso- and microhabitat requirements of fish is particularly relevant to the conservation of rare and threatened species because habitat fragmentation and loss are usually the major threats to species with limited distributions and restricted habitat requirements (Labbe and Fausch, 2000; Dudgeon et al., 2006), and habitat restoration is typically the first step in species’ recovery plans and river restoration in general (Bond and Lake, 2003). Fausch et al. (2002) believe that global efforts to conserve rare and endangered fish species have been hindered by the way ecologists have tended to study habitat use and requirements within only small fragments of the total river environment. They argue that the critical habitats for fish at various stages of their life history are often created and maintained by processes operating at higher spatial scales than the microhabitat level commonly studied, and these processes must be understood to guide management actions (Fausch et al., 2002).

This paper aims to document the macro-, meso- and microhabitat habitat associations of a small threatened Percichthyid, the Oxleyan pygmy perch, *Nannoperca oxleyana* Whitley, in eastern Australia. This species is listed as endangered by the IUCN (IUCN, 2004), by the Australian Society for Fish Biology and under the Australian Commonwealth Environment Protection and Biodiversity Conservation Act 1999 and New South Wales (NSW) Fisheries Management Act 1994. It is also listed as vulnerable under the Queensland Nature Conservation Act 1992. Habitat destruction, degradation and fragmentation and negative interactions with introduced species, are considered significant threats to *N. oxleyana* (Pusey
et al., 2004; NSW DPI, 2005). Recovery plans have been prepared for *N. oxleyana* with the overall aim of returning the species to a position of viability in nature (Arthington, 1996; NSW DPI, 2005). However, incomplete knowledge of the distribution and habitat requirements of the species and the main threatening processes has constrained the effectiveness of recovery actions (Pusey et al., 2004; NSW DPI, 2005).

Studies on the habitat requirements of *N. oxleyana* throughout its native range form part of two larger programmes of research designed to develop a sound ecological basis for the development of recovery actions (Arthington, 1996; Hughes et al., 1999; Knight, 2000, in press; Knight and Butler, 2004). In this study of habitat requirements historical distribution records have been collated and extensive surveys undertaken in different types of water bodies throughout and beyond the known range of this species in south-eastern Queensland and north-eastern NSW, Australia. In the more populated parts of the range of the species in northern NSW multivariate models of meso- and microhabitat use have been developed. The results of a more detailed spatial/temporal study of microhabitat use by one Queensland population are also reported, with particular emphasis on the importance of submerged aquatic macrophytes, a prominent feature of many sites supporting *N. oxleyana*. Using this information the habitat requirements of *N. oxleyana* across the three spatial scales are defined and the implications of our findings for the conservation of this species, and endangered fishes in general, are discussed.

**METHODS**

**Distribution and macrohabitat associations**

The area surveyed extended 900 km along the east coast of Australia (including offshore islands), from the northern extremity of Fraser Island and the adjacent mainland Mary River catchment in Queensland south to the Myall River catchment in NSW (24° 52’S - 32° 38’S; Figure 1). This narrow belt of lowland country lying between the coast and the coastal ranges is part of the coastal lowlands (‘wallum’) ecosystem of south-eastern Queensland and north-eastern NSW. Wallum country is characterised by *Banksia*-dominated heath vegetation growing on siliceous (quartz dominated) sands (Griffith et al., 2003). It has a seasonally distributed annual rainfall (1016-1778 mm) and freshwater lakes, creeks and wetlands are prominent landscape features.
Figure 1. Distribution of *Nannoperca oxleyana* (white circles) within a) and b) the study area in Australia, c) south-eastern Queensland and d) north-eastern New South Wales. Catchments inhabited by the species are shaded and historical records of the species before 1990 where it was not subsequently re-captured are depicted (black circles). Catchment, locality and water body names referred to in the text are given here.
Surveys of 261 localities were undertaken in south-east Queensland between 1992 and 1998 and of 304 water bodies in north-eastern NSW between June 2000 and September 2004. Owing to anthropogenic habitat fragmentation and the natural intermittent connection of some coastal water bodies, tributary streams and lentic bodies within larger discrete drainage systems were treated as separate water bodies. In both Queensland and NSW, in addition to surveying many water bodies that had never been studied, all localities recorded as supporting *N. oxleyana* before 1992 (Queensland) and 2000 (NSW) were re-sampled in an attempt to ascertain population persistence. To provide a comprehensive analysis, locality records collected between 1973 and 2006 by the authors and other researchers were also examined for positive records of *N. oxleyana* (see Pusey et al. 2004 for summary and maps of Queensland rivers surveyed, and NSW Department of Primary Industries’ [DPI] Aqua-See Database [available at: www.bionet.nsw.gov.au]).

Sampling methods for *N. oxleyana* are detailed in Knight et al. (2007). Briefly, techniques included the deployment of 9-40 collapsible unbaited fish traps (250 x 250 x 450 mm, 3 mm mesh) soaked for 15-30 min at 1.5-2.0 m intervals on the substrate and either 2-5 shots with a seine net (4 x 1.5 m, 2.5 x 1.5 m or 1.5 x 1 m, 2-5 mm mesh) or 10 minutes of backpack electrofishing (Smith-Root model 12B electrofisher) (Marine Navaid, Botany, NSW, Australia). 15 x 2 minute operations with a boat electrofisher (2.5 m aluminium punt fitted with a 2.5 kW Smith-Root model GPP 2.5 H/L generator) were undertaken in two large lakes. Dip netting was also employed at several sites. Following capture and identification, most fish were released at their place of capture, although on occasion, voucher specimens from Queensland and NSW were sent to the Queensland Museum, Brisbane and the Australian Museum, Sydney, respectively.

Site locations were recorded with a Garmin 12 GPS (Whitworth’s Marine & Leisure, Caringbar, NSW, Australia) and data on elevation and distance from the coastline were derived from 1:25 000 topographic maps (Pusey et al., 2004). At each site, the surrounding soil type was recorded and riparian and aquatic vegetation was identified to species level. Water quality data including temperature (°C), dissolved oxygen (mg L⁻¹), pH, conductivity (µS cm⁻¹) and turbidity (NTU) were recorded using either a Horiba U10 (Australian Scientific, Newcastle, NSW, Australia) or a red spirit thermometer, a YSI Model 57 oxygen meter (Yellow Springs Instruments Company, Inc., Yellow Springs, Ohio, USA), a TPS LC80 (TPS Pty Ltd, Springwood, Brisbane, Queensland, Australia) or Suntex TS-1 pH/mv meter (Suntex Instruments Co., Ltd., Hsi-Chih City, Taipei County, Taiwan), a YSI Model 33 SCT conductivity meter (Yellow Springs Instruments Company, Inc., Yellow Springs, Ohio, USA), and a Hach Turbidimeter Model 16800 (Ecotech Pty Ltd., Brisbane Branch Office, Brisbane,
Australia). Water colour was visually assessed and classified as clear, light tannin, medium tannin, dark tannin, translucent/cloudy, or heavy suspended solids (Arthington, 1996).

**Meso/microhabitat use in NSW**

Research into the meso- and microhabitat use patterns of *N. oxleyana* was undertaken in NSW in conjunction with distribution surveys near Evans Head (Figure 1; Knight, 2000). Four lotic mesohabitats were identified: riffles, runs, pools, and backwaters. Their distinguishing characteristics included channel morphology, gradient and current velocity. Depending upon availability, each mesohabitat type was sampled at three sites in each creek, giving a maximum of 12 sites per creek. Three microhabitat types were identified within a mesohabitat, including beds of aquatic vegetation (sedges and macrophytes), open water (areas with no significant vegetation), and steeply shelving or undercut banks typically fringed with the semi-submerged branches and fine rootlets of riparian vegetation such as Coral fern *Gleichenia dicarpa* or *Baloskion* (ex. *Restio* *tetraphyllum*). Three unbaited traps were set for 15 min on the substrate of each microhabitat present within a site. Lentic systems were sampled in a similar way. Given the absence of mesohabitat units, a maximum of three sites was chosen per lentic water body based on accessibility, the successful deployment of sampling gear and the presence of aquatic vegetation adjacent to an area of open water. Lentic systems in NSW lacked steep/undercut bank microhabitats. In total, 131 microhabitats within 76 mesohabitat sites within 21 lotic systems and 115 microhabitats within 62 sites within 22 lentic systems were sampled between June and September 2000.

Creek mesohabitat complexity was quantified and partitioned into a physical and a cover habitat component based on methods developed by Pusey *et al.* (1993, 2000). Physical components included depth, current velocity and substrate characteristics. Average depth (± 0.5 cm) was calculated by averaging three measurements made throughout the site. Current velocity (m s$^{-1}$) was recorded with a Universal Current Velocity Meter, Model OSS-B1 (Hydrological Services, Sydney, NSW, Australia). In sites with depths less than 0.75 m, average velocity was recorded at 0.6 of the distance from the surface to the substratum, and at depths greater than 0.75 m, velocity was recorded at 0.2 x and 0.8 x water column depth (after Bovee and Milhous, 1978). Substrate composition was visually estimated as the proportion of mud (<1 mm diameter), sand (1-16 mm), fine gravel (16-32 mm), gravel (32-64 mm), cobble (64-128 mm), rock (128-512 mm) or bedrock/‘coffee’ rock (>512 mm) present per site. Coffee rock is a soft sandy rock cemented with organic matter (see Chapman and Murphy, 1991). Cover components including the extent of understorey riparian cover, and the
abundance of aquatic vegetation and leaf litter were estimated as the percentage cover per site. The extent of steeply shelving or undercut bank was expressed as a proportion of wetted channel perimeter. Woody debris was expressed as the number of pieces per metre of wetted channel perimeter.

**Analysis of NSW meso/microhabitat use**

Relationships between *N. oxleyana* catch rates recorded near Evans Head, NSW and the lotic mesohabitat and lotic and lentic microhabitat parameters were modelled with multilevel Poisson regression (Extra Poisson, first-order PQL estimates) using the software Multilevel Modelling for Windows (MLwiN 1.1; Rasbash *et al*., 2000). Systematic variation was tested within a hierarchical sampling structure which included water bodies, sites within water bodies and observations within sites. The models predicted the log probability of the dependent variable, catch-per-trap, varying in relation to independent predictors. Given high multicollinearity between the habitat predictors in the mesohabitat model, Principal Component Analysis (PCA) with Varimax rotation was performed separately on the continuous physical and cover habitat parameters. Scores for each creek site on each principal component were calculated and used as independent variables in the multilevel model. For the microhabitat model, dummy variables were incorporated to represent the three microhabitat types. The model outputs included a calculated partial coefficient (Cf.) and the standard error (S.E.) of each predictor. The significance of an effect parameter, which estimated the difference between a pair of predictors, was determined by the Wald statistic: Wald = Cf./S.E. The Wald statistic was referred to the standard normal distribution so that statistics greater than or equal to 1.96 were two-tailed significant to *P*<0.05. Significant differences between predictors in the microhabitat model were tested using pairwise comparisons. The Wald statistic was squared and compared with the chi-squared distribution with one degree of freedom so that statistics ≥3.84 were two-tailed significant to *P*<0.05. *P*-values were adjusted for multiple comparisons using the Bonferroni procedure. For further details on multilevel modelling refer to Snijders and Bosker (1999).

**Microhabitat use in Queensland**

Microhabitat studies were undertaken in Spitfire Creek, a coastal wetland and creek draining to the east coast of Moreton Island, south-eastern Queensland (Figure 1). Unbaited traps were used to examine the relative prevalence of *N. oxleyana* in three types of microhabitat: beds of the submerged sedge *Eleocharis ochrostachys*, areas with a combination of other plant species
Distribution and habitat associations of the endangered Oxleyan pygmy perch

such as *Juncus*, *Triglochin* and *Nymphaea*, and areas with no significant vegetation (termed ‘open water’). These microhabitat types differed in their character and structural complexity. *Eleocharis ochrostachys* grew in shallow water forming dense beds consisting of masses of slender submerged and some emergent stems (35 cm long, 1-1.2 mm thick) whereas *Juncus*, *Triglochin* and *Nymphaea* formed more diffuse beds of vegetation within which plant stems, submerged foliage and accumulated free plant debris provided some structural habitat. The open water areas were selected to provide sites with as little aquatic vegetation as possible. Each trapping site had similar depth characteristics (maximum depth 1 m) and very low to no current velocity during trapping sessions. Traps were set for 15 min on wooden stakes driven into the substrate with two ‘surface’ traps set at 20 cm below the water surface and two ‘deep’ traps set near the substrate. Two replicates of each of the three habitat types were chosen from among the patches at the study site and traps were cleared twice a day (8am and 4pm). This entire design was repeated on the following day giving a total of 96 trap catch values over the two-day period. At the end of each 15 min trapping session all individuals were removed, counted and released immediately. This design was repeated nine times over a period of one year (April 1994 - March 1995) to determine seasonal trends in microhabitat use.

**Analysis of Queensland microhabitat use**

Two data sets were derived from the original fish trap data. Dataset A represents fish catches from six occasions between April and November 1994 (i.e. approximately monthly excluding June and July), and dataset B represents fish catches from three occasions during 1994/1995 (December, February and March). There were many zero and low trap catches, therefore catches from each pair of traps at each water depth and time of day were pooled and the data were fourth-root transformed in order to achieve near normality of distribution. For traps set from April to November 1994 (dataset A) the use of parametric statistical analysis was constrained by high variance attributable to low catches from ‘other macrophytes’. This habitat type was removed from the analysis and a four-way ANOVA performed on the 1994 data from *Eleocharis ochrostachys* beds and open water. For traps set from December to March (dataset B) there was sufficient data to examine differences in catch among the three habitat types by means of a four-way ANOVA.
RESULTS

Distribution and macrohabitat associations

*Nannoperca oxleyana* was captured during recent surveys, or recorded in past surveys, from 33 water bodies in south-eastern Queensland and 57 water bodies in north-eastern NSW (Figure 1). In conjunction with information sourced from the NSW Department of Primary Industries’ [DPI] Aqua-See Database, published literature and unpublished data, these survey records reveal that since 1990, *N. oxleyana* has been documented from 105 water bodies within 75 discrete drainage systems within five mainland catchments and on three offshore islands (Table 1), with 72% of the discrete systems located in NSW. The species was not detected from four water bodies initially known to support the species prior to 1990 (Figure 1). A total of 63% of records came from lotic water bodies (Table 1) with the species found mostly within small tributary streams but occasionally, in Queensland, within the main river channel (e.g. Noosa River). Whereas equivalent numbers of records came from lotic and lentic water bodies in NSW, only three records involve lentic water bodies in Queensland (lakes on Moreton Island).

In NSW, all *N. oxleyana* water bodies were located within 8 km of the coastline at elevations of 30 m or less above mean Australian sea level. In mainland Queensland, most water bodies supporting this species were also in close proximity to the coastline. Exceptions included two localities in the Mary River catchment (27 km inland) and three localities in the Maroochy River catchment (14 km inland). In mainland Queensland the species typically occurred in small shallow tributaries within catchments of 58-687 km² in area. These low gradient tributaries (mean: 0.05%; range: 0.01-0.09%) were located at elevations of 4-40 m (mean: 18.0 m) above mean Australian sea level and at distances of 7-123 km (mean: 70 km) from the river mouth and 15-54 km (mean: 32 km) from the river source (Queensland data extracted from Pusey et al., (2004)).

Most systems inhabited by *N. oxleyana* drained through coastal ‘wallum’ (*Banksia*-dominated heath) ecosystems over siliceous sands in dune valleys and swales. *Melaleuca quinquenervia, Banksia ericifolia, Banksia aemula, Callisemon spp., Gleichenia dicarpa, Baloskion tetraphyllum* and a variety of other heath species commonly formed riparian communities, while emergent and aquatic vegetation such as *Philydrum lanuginosum, Lepironia articulata, Gahnia* sp., *Eleocharis ochrostachys* and other *Eleocharis* species, *Triglochin* sp., *Chara* sp. and *Sphagnum falcatulum* often proliferated in streams, lakes and swamps. Exceptions included three un-named NSW lotic systems in the Clarence River catchment south of Evans Head, which drained through a complex of tall woodland forest and
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either wallum scrub or *M. quinquenervia* swamp and had a compound substratum comprised of sand and clay. An additional nearby un-named creek and Little Canalpin Creek on North Stradbroke Island, Queensland flowed through a swamp complex dominated by *M. quinquenervia, Eucalyptus robusta* and an assemblage of littoral rainforest species growing on either grey acid soils or peaty soils. However, all five tributaries originated within or eventually drained into wallum environments and contained similar aquatic vegetation communities.

Table 1. Number of *Nannoperca oxleyana* localities documented since 1992 in Queensland and since 2000 in NSW. Tributary streams and lentic bodies within larger discrete drainage systems were treated as separate water bodies.

<table>
<thead>
<tr>
<th>Location</th>
<th>River/tributary</th>
<th>Lake/pond/dam</th>
<th>Swamp</th>
<th>Discrete drainage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Queensland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mary River Catchment</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Noosa River Catchment</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Maroochy River Catchment</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Fraser Island</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Moreton Island</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>North Stradbroke Island</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>NSW</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richmond River Catchment</td>
<td>23</td>
<td>12</td>
<td>9</td>
<td>31</td>
</tr>
<tr>
<td>Clarence River Catchment</td>
<td>11</td>
<td>5</td>
<td>10</td>
<td>23</td>
</tr>
</tbody>
</table>

Waters occupied by *N. oxleyana* in both Queensland and NSW were almost always fresh and acidic (Table 2). pH never exceeded 6.9 and conductivity at all but one site (Coondoo Creek, Queensland) never exceeded 830 µS cm\(^{-1}\). Thirty-two sites occupied by the species had a pH of less than 4.0 and one site in an un-named lake northwest of Evans Head, NSW, had a pH of 3.32. Mean dissolved oxygen saturation levels were higher in sites supporting *N. oxleyana* than in all sites sampled and were never less than 20.2% (2.15 mg L\(^{-1}\)). Minimum, maximum and mean water temperatures were similar between all sites sampled and *N. oxleyana* sites. Waters in *N. oxleyana* habitats ranged from clear to dark tannin stained, were never cloudy, and lacked heavy suspended solids. Most *N. oxleyana* sites had low turbidity levels with only four sites having turbidity levels greater than 13 NTU. Additional data on the water quality associations of *N. oxleyana* were provided by sampling along an environmental gradient in Mara Creek, north-eastern NSW (Table 2). This creek intermittently connects to
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the ocean but at the time of sampling (18 September 2002) the mouth was closed and the lower reaches contained anoxic saline water (Table 2). A salt wedge dissipated upstream and waters within the middle reaches and headwaters of this creek were less than 200 µS cm\(^{-1}\) conductivity (i.e. fresh). Although the shallow headwaters of this creek were also anoxic the waters were progressively aerated as they flowed over tree roots and coffee rock towards the creek’s middle reaches. Despite intensive sampling in all three reaches, \(N. \text{ oxleyana}\) was captured only within the middle reaches where waters were fresh and had a dissolved oxygen saturation level of 29.6% (2.76 mg L\(^{-1}\)).

Table 2. Physicochemical data for study sites sampled for \(Nannoperca \text{ oxleyana}\) in Queensland and NSW (n = 333), and for sites supporting \(N. \text{ oxleyana}\) in Queensland (n = 15) and in NSW (n = 83) with Mara Creek presented as an exception.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All sites(^{a})</th>
<th>(N. \text{ oxleyana}) sites(^{a})</th>
<th>Mara Creek</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E.</td>
<td>Range</td>
<td>Lower Reach</td>
</tr>
<tr>
<td>Water temp. (ºC)</td>
<td>16.2 ± 0.21</td>
<td>9.6-31.1</td>
<td>16.1 ± 0.34</td>
</tr>
<tr>
<td>Dissolved oxygen:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg L(^{-1}))</td>
<td>5.67 ± 0.123</td>
<td>0.04-14.09</td>
<td>6.42 ± 0.189</td>
</tr>
<tr>
<td>(%)</td>
<td>58.5 ± 1.37</td>
<td>0.4-167.4</td>
<td>65.8 ± 2.06</td>
</tr>
<tr>
<td>pH</td>
<td>4.82 ± 0.060</td>
<td>3.25-8.27</td>
<td>4.47 ± 0.087</td>
</tr>
<tr>
<td>Cond. (µS cm(^{-1}))</td>
<td>886 ± 210.3</td>
<td>34-31400</td>
<td>186 ± 22.7</td>
</tr>
<tr>
<td>Turb. (NTU)</td>
<td>18 ± 2.3</td>
<td>0-160</td>
<td>14 ± 3.6</td>
</tr>
</tbody>
</table>

\(^{a}\) Sample sizes include sites sampled within water bodies but exclude desiccated sites. Also excludes data for a number of Queensland sites as these data are unavailable.

Mesohabitat use

In total, 55 fish were captured from 27 of 76 mesohabitat sites sampled in 21 lotic systems near Evans Head, NSW. Pools were the predominant mesohabitat sampled with only two creeks displaying mesohabitat heterogeneity. Sixty-one pools, six backwaters, six runs and three riffles were sampled; however, \(Nannoperca \text{ oxleyana}\) was only captured in pools and backwaters. Mean CPUE was 0.16±0.04 and 0.14±0.09 fish/trap/15 min for pools and backwaters, respectively. The species was captured in shallow mesohabitats with a predominately sandy substrate and a depth of 0.5 m, where current velocities averaged 0.02 m s\(^{-1}\) and did not exceed 0.3 m s\(^{-1}\) (Table 3). Beds of aquatic vegetation and leaf litter were
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common and typically covered 38% and 34%, respectively, of the substrate of mesohabitats supporting *N. oxleyana*. Woody debris, overhanging vegetation and steep/undercut banks were present in 26%, 58% and 22%, respectively, of all sites sampled but were recorded at higher frequencies and densities in *N. oxleyana* sites (Table 3).

Table 3. Mesohabitat parameters of creek sites supporting *Nannoperca oxleyana* near Evans Head, NSW.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N. oxleyana sites (n = 27)</th>
<th>All sites (n = 76)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Freq.</td>
<td>Min.</td>
</tr>
<tr>
<td>Current velocity (m s(^{-1}))</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>100</td>
<td>0.21</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>Mud/detritus (%)</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Coffee rock (%)</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Aquatic Vegetation (%)</td>
<td>85</td>
<td>0</td>
</tr>
<tr>
<td>Leaf Litter (%)</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>Woody debris (#/m)</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Overhanging veg. (%)</td>
<td>74</td>
<td>0</td>
</tr>
<tr>
<td>Steep/undercut bank (%)</td>
<td>41</td>
<td>0</td>
</tr>
</tbody>
</table>

PCA found strong correlations among the physical and cover habitat variables. For the physical habitat parameters, 79% of the variation in the data set could be explained by two components. The first component related to proportion of coffee rock (+0.96) and current velocity (+0.96). The second component related to mud/detritus (+0.93), depth (+0.37) and sand (-0.92). For the cover habitat parameters, two components explained 67.3% of the variation. Component one for the cover habitat variables had high positive loadings on steep/undercut banks, overhanging vegetation and open water (the inverse of percentage cover of aquatic vegetation). However, given the results of the microhabitat analysis (see below), different abundances of fish would be expected to be associated with structural cover provided by steep/undercut banks and overhanging vegetation and open water. Therefore, percentage of open water was excluded from the PCA and included in the multilevel model as an independent variable. Loadings for the remodelled cover habitat parameters differed little from the initial analysis. Two components explained 76% of the variance. Component one
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related to steep/undercut banks (+0.91) and overhanging vegetation (+0.77) and component
two related to leaf litter (+0.94) and woody debris (+0.66).

The scores for each site on each component were used as independent variables in the
multilevel Poisson regression model (Table 4). Significant differences were detected for all
PCA-transformed habitat variables except for coffee rock and current velocity. The log of the
predicted abundances of *N. oxleyana* caught in traps significantly increased as creek
mesohabitats were comprised of increasing proportions of sandy substrates, leaf litter and
woody debris, steep/undercut banks and overhanging vegetation, decreasing proportions of
mud/detritus substrates and decreasing depth (Figure 2). A positive relationship also existed
between an increase in the log probability of catching *N. oxleyana* and increasing percentage
cover of aquatic vegetation. A significant amount of unexplained variation was detected at the
random site level. The extra Poisson variance was also significant, thereby indicating that the
catch data were over dispersed. This clumping suggests that catches were density dependant
(see Knight *et al.*, 2007, for a detailed explanation).

Table 4. Parameter estimates for multilevel Poisson regression analysis of creek mesohabitat use
by *Nannoperca oxleyana* near Evans Head, NSW. PCA transformed habitat variables were
modelled. Aquatic vegetation was also modelled as an independent variable.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Cf.</th>
<th>S.E.</th>
<th>Wald</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-4.298</td>
<td>0.585</td>
<td>-7.347 *</td>
</tr>
<tr>
<td>PPC1: Coffee rock and current velocity</td>
<td>0.386</td>
<td>0.342</td>
<td>1.129</td>
</tr>
<tr>
<td>PPC2: Mud/detritus and depth; not sand</td>
<td>-1.206</td>
<td>0.399</td>
<td>-3.023 *</td>
</tr>
<tr>
<td>CPC1: Steep/undercut banks and O.H. veg.</td>
<td>0.803</td>
<td>0.278</td>
<td>2.888 *</td>
</tr>
<tr>
<td>CPC2: Leaf litter and woody debris</td>
<td>0.776</td>
<td>0.327</td>
<td>2.373 *</td>
</tr>
<tr>
<td>Aquatic vegetation; not open water</td>
<td>0.020</td>
<td>0.010</td>
<td>2.000 *</td>
</tr>
</tbody>
</table>

| Random effects                              |        |        |         |
| Water body                                  | 0.958  | 0.765  | 1.252   |
| Site                                        | 1.993  | 0.740  | 2.693 * |

| Extra Poisson variance                      | 0.342  | 0.026  | 13.154 *|

PPC = physical habitat principal component. CPC = cover habitat principal component. O.H.
Veg. = overhanging vegetation. Wald Statistics ≥1.96 were two-tailed significant to *P*<0.05. * indicates a significant difference.
Microhabitat use

In total, 59 and 62 beds of aquatic vegetation were sampled in the respective lotic and lentic systems surveyed near Evans Head, NSW. Areas of open water were sampled in lotic systems on 55 occasions and in lentic systems on 53 occasions. Steep/undercut banks fringed with the semi-submerged branches and fine rootlets of riparian vegetation occurred only in lotic systems and were sampled on 17 occasions. Trap catches differed significantly among the microhabitat types within both lotic and lentic systems (Table 5). Significantly higher abundances of *N. oxleyana* were caught in aquatic vegetation than in open water in both lotic ($\chi^2 = 37.381, P<0.001, \alpha = 0.017$, Bonferroni adjusted) and lentic ($\chi^2 = 33.339, P<0.001, \alpha = 0.05$) systems. Creek catches from bank habitat were also significantly higher than from open water areas ($\chi^2 = 47.701, P<0.001, \alpha = 0.017$, Bonferroni adjusted), but not so from aquatic vegetation ($\chi^2 = 4.060, P = 0.044, \alpha = 0.017$, Bonferroni adjusted). Significant variation was also detected at the random site level in the lentic systems model and at the water body level in both models (Table 5), indicating that variation of certain unmeasured parameters among water bodies and sites within water bodies (e.g. substrate type, percentage coverage of leaf litter) was contributing to variations in the catch rates. In both models, the extra Poisson variance was also significant, suggesting that catches were density dependant.
Table 5. Parameter estimates for multilevel Poisson regression analysis of microhabitat use by *Nannoperca oxleyana* near Evans Head, NSW.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Lotic systems</th>
<th>Lentic systems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cf.</td>
<td>S.E.</td>
</tr>
<tr>
<td>Fixed effect</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept (Open water)</td>
<td>-4.987</td>
<td>0.534</td>
</tr>
<tr>
<td>Open Water vs Aquatic veg.</td>
<td>1.877</td>
<td>0.307</td>
</tr>
<tr>
<td>Open Water vs Bank</td>
<td>2.293</td>
<td>0.332</td>
</tr>
<tr>
<td>Random effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water body</td>
<td>2.645</td>
<td>1.381</td>
</tr>
<tr>
<td>Site</td>
<td>2.232</td>
<td>0.779</td>
</tr>
<tr>
<td>Extra Poisson variance</td>
<td>0.271</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Wald statistics ≥1.96 were two-tailed significant to *P*<0.05. * indicates a significant difference.

At a finer spatial scale in Spitfire Creek (Moreton Island) there were spatial and temporal differences in *N. oxleyana* catches related to water depth, time of day, habitat type and season. Between April and November 1994 there were significant effects for water depth, time of day and month but not for habitat type (Table 6). However, during the warmest months (December, February and March) there were significant effects for habitat type as well as depth (Table 7). In this case *N. oxleyana* was more abundant in the traps set near the substrate within beds of *E. ochrostachys* than in beds of other macrophytes and traps set in open water areas. There were also significant interactions between depth of trapping and month (*P*<0.001) and depth and time of day (*P*<0.05) (Table 7). The two datasets combined show the seasonal trends in total catches (based on equivalent trapping effort) of *N. oxleyana* according to microhabitat type, water depth and time of day (Figure 3). It is apparent that total numbers caught increased in summer months (December to March) and that habitat use by *N. oxleyana* varied with time of year, the strongest pattern being the association of this species with *E. ochrostachys* beds in the summer months and a more diffuse pattern of use of other habitat types during the rest of the year (Figure 3).
Table 6. Significant differences in numbers of *Nannoperca oxleyana* trapped in Spitfire Creek, Moreton Island, Queensland between April and November 1994, based on a four-way ANOVA.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>5</td>
<td>5.257 ***</td>
</tr>
<tr>
<td>Depth</td>
<td>1</td>
<td>44.418 ***</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>8.501 **</td>
</tr>
<tr>
<td>Microhabitat</td>
<td>1</td>
<td>0.321</td>
</tr>
<tr>
<td>Month x Depth</td>
<td>5</td>
<td>2.004</td>
</tr>
<tr>
<td>Month x Time</td>
<td>5</td>
<td>1.348</td>
</tr>
<tr>
<td>Month x Microhabitat</td>
<td>5</td>
<td>1.808</td>
</tr>
<tr>
<td>Depth x Time</td>
<td>1</td>
<td>0.003</td>
</tr>
<tr>
<td>Depth x Microhabitat</td>
<td>1</td>
<td>0.005</td>
</tr>
<tr>
<td>Time x Microhabitat</td>
<td>1</td>
<td>2.516</td>
</tr>
<tr>
<td>Month x Depth x Time</td>
<td>5</td>
<td>0.877</td>
</tr>
<tr>
<td>Month x Depth x Microhabitat</td>
<td>5</td>
<td>1.167</td>
</tr>
<tr>
<td>Month x Time x Microhabitat</td>
<td>5</td>
<td>1.011</td>
</tr>
<tr>
<td>Depth x Time x Microhabitat</td>
<td>1</td>
<td>3.360</td>
</tr>
<tr>
<td>Month x Depth x Time x Microhabitat</td>
<td>5</td>
<td>0.477</td>
</tr>
<tr>
<td>Error</td>
<td>144</td>
<td></td>
</tr>
</tbody>
</table>

* = P<0.05, ** = P<0.01, *** = P<0.001.

Table 7. Significant differences in numbers of *Nannoperca oxleyana* trapped in Spitfire Creek, Moreton Island, Queensland between December 1994 and March 1995, based on a four-way ANOVA.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>2</td>
<td>1.434</td>
</tr>
<tr>
<td>Depth</td>
<td>1</td>
<td>9.102 **</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>1.743</td>
</tr>
<tr>
<td>Microhabitat</td>
<td>2</td>
<td>8.747 ***</td>
</tr>
<tr>
<td>Month x Depth</td>
<td>2</td>
<td>8.582 ***</td>
</tr>
<tr>
<td>Month x Time</td>
<td>2</td>
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<td>Month x Microhabitat</td>
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<td>4</td>
<td>1.255</td>
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<tr>
<td>Error</td>
<td>96</td>
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* = P<0.05, ** = P<0.01, *** = P<0.001.
Figure 3. Total numbers of *Nannoperca oxleyana* collected from all traps set at two water depths in each of three microhabitat types: a) *Eleocharis ochrostachys*, b) other macrophytes, c) open water, over 9 months in 1994/1995. Solid black bars represent morning catches from all months, grey bars represent evening catches from April to November 1994, and hatched bars represent evening catches from December 1994 to March 1995. Total catches per habitat type in surface and deeper traps are given in the top left corner of each box. Tables 6 and 7 show significant differences in catches and habitat use during April to November 1994, and December 1994 to March 1995, respectively, based on these data sets.
DISCUSSION

The habitat associations of rare and endangered fishes are of supreme interest to scientists and managers intent upon conserving the remaining populations and/or restoring habitat conditions that will support viable populations. Often, critical habitats are created and maintained by processes operating at higher spatial scales than the microhabitat level commonly studied in river systems (Fausch et al., 2002). Relatively few studies have examined the importance of fish habitat at multiple scales simultaneously (Pusey et al., 1993, 1995, 2000; Labbe and Fausch, 2000; Crook et al., 2001; Bond and Lake, 2003), a necessary approach when the objective is to advise on management actions to protect or restore an endangered species threatened by multiple disturbances operating across multiple spatial scales. In this study of an endangered fish species, the Oxleyan pygmy perch, *Nannoperca oxleyana*, we have explored the distribution and macro-, meso- and microhabitat use patterns of the species as part of broader efforts to guide recovery actions for this species in Queensland and New South Wales (NSW), Australia. Here major findings and their implications for the conservation of *N. oxleyana* are discussed, and from this a set of principles is proposed for habitat investigations to support the conservation of any endangered fish species.

At the macrohabitat scale, *N. oxleyana* is confined primarily to dystrophic, acidic, freshwater systems draining through sandy coastal lowlands (the ‘wallum’ or *Banksia*-dominated heath ecosystems) along approximately 530 km of coastline from Coongul Creek on Fraser Island, south-eastern Queensland (25º 16’S, 153º 09’E) south to Tick Gate Swamp near the township of Wooli, north-eastern NSW (29º 54’S, 153º 15’E). Two features of the distribution of this species are particularly interesting – the break between the NSW and Queensland populations, and the far greater prevalence of *N. oxleyana* populations in northern NSW than in the equivalent length of coastline in Queensland.

It would appear that human activities have had a marked influence on contemporary species presence/absence patterns and may have been responsible for the species’ current southern distributional limits and more prominent gaps within this distribution. Indeed, large expanses of habitat suitable for *N. oxleyana*, particularly within the distribution gap, which stretches approximately 250 km from the Glasshouse Mountains in Queensland southward to the township of Broadwater in NSW (Figure 1), have been destroyed, fragmented or degraded by residential and resort development, road construction, agriculture, forestry, sand mining and water pollution (Arthington, 1996; Graham, 2004a, b; Pusey et al., 2004; NSW DPI, 2005; Knight, in press). There is also strong evidence of a southern range contraction in the last 30 years as targeted sampling of the area from Tick Gate Swamp south to and beyond Cassons
Creek (where the species was collected in 1976) failed to detect the species. Furthermore, the species was not recorded from a number of other localities where it was collected between 1929 and 1976, including Beerwah Forest in Queensland, and Bookram Creek, and an unnamed water body near Coraki, in NSW. With the exception of Bookram Creek, these localities have been heavily affected by agricultural and/or forestry activities and presumably no longer provide adequate habitat and environmental conditions to support *N. oxleyana*. Likewise, the distribution of the species in undisturbed streams near the Richmond River in NSW comes to an abrupt halt in degraded downstream sections modified into sugar cane drains.

Human disturbances may take effect across the full range of habitat scales assessed in this study. For example, draining of low-lying swampy areas can result in the destruction of entire perched lakes, swamps and connected tributaries (Timms, 1977, 1986). The low nutrient waters of wallum lakes, creeks and swamps may also be easily degraded by excess nutrients, toxic substances and silt entering via urban, agricultural and industrial runoff, and by recreational and camping activities (Timms, 1986; Outridge et al., 1989; Pusey et al., 2004). Likewise, localised riparian and littoral vegetation clearing may lead to the rapid erosion of sandy substrates followed by siltation and infilling of pools and smothering of important microhabitats such as macrophyte beds (Arthington, 1996; Knight, 2000; Pusey and Arthington, 2003). Several Queensland coastal streams that could be expected to provide habitat for *N. oxleyana*, but do not, are clogged with dense overhanging swards of introduced para grass, *Urochloa mutica*. Infestation by this semi-aquatic species can reduce light penetration, smother native macrophytes, degrade water quality and aquatic habitat (Arthington et al., 1983; Pusey and Arthington, 2003), and bring about associated changes in food web structure (Bunn et al., 1997). These threatening processes frequently coincide and interact in disturbed coastal catchments and may lead to the extirpation of entire populations of this endangered species.

The second feature of special interest is that populations of *N. oxleyana* appear to be far more prevalent in northern NSW than in the equivalent length of coastline in Queensland. The prevalence of *N. oxleyana* in many northern NSW water bodies, and particularly north of the Clarence River, may be attributed to interactions between hydrology and landscape features such as floodplain development and the connectivity potential of drainages spread across the expansive low lying coastal plains. Intermittent connection among water bodies during high rainfall events or large floods emanating from the Richmond and Clarence Rivers (Knight, 2000, in press) may facilitate the dispersal of *N. oxleyana*, thereby allowing the species to colonise new systems and/or to recolonise previously disturbed areas (Hughes et al., 1999; Knight, 2000; Pusey et al., 2004). Flood dispersal and colonisation of suitable habitats may also
explain the higher association of *N. oxleyana* with lentic habitats in NSW than in Queensland, as numerous lakes (both natural and artificial), swamps and small dams are distributed across the floodplains north of the Clarence River.

Within Queensland, *N. oxleyana* is currently known from a total of 21 discrete water bodies with only six of these located in isolated creeks on the mainland. Hence most populations are separated by relatively large land distances and stretches of ocean and they cannot be connected by flooding (see Figure 1). Genetic analysis (based on mitochondrial and allozyme methods) of nine isolated mainland and insular populations has revealed high levels of genetic differentiation, implying that these isolated populations have diverged from each other as a result of extremely limited dispersal (Hughes *et al.*, 1999). Likewise, the extent of the geographic gap between mainland populations in Queensland and NSW (~250 km) implies that natural gene flow between the two areas is severely restricted at the present time. In contrast, the NSW coastal floodplains north of the Clarence River could be inhabited by one or several dispersed and genetically distinctive sub-populations of *N. oxleyana* distributed across a number of intermittently connected water bodies. The genetic structure of the NSW populations is currently being investigated to test this proposition and to assist in developing and prioritising management actions based on a sound understanding of population structure and the most appropriate foci for conservation of genetically differentiated populations throughout the entire range of this species (see Arthington, 1996; Hughes *et al.*, 1999; Page *et al.*, 2004; NSW DPI, 2005 for discussion).

The meso- and microhabitat associations of *N. oxleyana* reveal affinities that are particularly relevant to its conservation. Although both lotic and lentic environments provide habitat for *N. oxleyana*, a defining characteristic among the inhabited sites was a distinct lack of stream flow. The species was found only in slow flowing pools and backwaters of river channels and tributaries as well as in swampy drainages and lakes, ponds and dams. This has implications for habitat protection/management in riverine localities where natural flow variability and/or changes in the flow regimes of regulated streams and rivers may create both low flow and high flow disturbances (Arthington and Pusey, 2003). Unnaturally low flow levels (e.g. caused by pumping or an upstream weir or impoundment) have the potential to deprive low flow and backwater habitats, and interconnected lakes, of sufficient water, whereas water releases (e.g. for irrigation purposes) may degrade microhabitat structure via bank erosion, and by scouring or removal of important structural elements such as aquatic vegetation (Bunn and Arthington, 2002; Arthington and Pusey, 2003; Mackay *et al.*, 2003).

At the microhabitat scale, positive relationships between fish presence/abundance and attributes of aquatic habitat structure and heterogeneity, such as those observed here for *N.*
Distribution and habitat associations of the endangered Oxleyan pygmy perch

Oxleyana, are well documented in streams, rivers, lakes and floodplain systems (Savino and Stein, 1989; Gelwick and Matthews, 1990; Pusey et al., 1993, 2000, 2004; Humphries, 1995; Bond and Lake, 2003; Arthington et al., 2005). Vegetated habitats provide small fishes with shelter and refuge from avian and aquatic predators and high flow conditions, as well as suitable resting, feeding and spawning grounds (Werner et al., 1983; McIvor and Odum, 1988; Wager, 1992; Pusey et al., 1993). Structural in-stream cover may reduce the impact of short periods of high flow with the power to disrupt spawning activities, displace eggs and small individuals downstream or carry fish into open areas with little protective cover (Milton and Arthington, 1985; Pusey et al., 1993, 2004). The significant, year-round association of *N. oxleyana* with deeper water, especially in beds of submerged sedges, may be a reflection of foraging activities focused on microcrustaceans, shrimps and aquatic insects associated with plants and the benthos (Pusey et al., 2004). When present, undercut banks, leaf litter and woody debris also provide cover for *N. oxleyana* in streams. Other members of the genus *Nannoperca* show a preference for habitat with low flows and dense in-stream cover in the form of large woody debris and macrophyte beds (Pen and Potter, 1991; Humphries, 1995; Allen et al., 2002). We conclude that the maintenance of natural stream bank and habitat structure and patterns of aquatic plant growth in these relatively fragile, sand bed coastal streams must be a high priority when developing principles and actions for catchment management and fauna conservation.

Given the extremely patchy occurrence of *N. oxleyana* across a range of vulnerable meso- and microhabitat types and the vulnerability of physically and genetically isolated populations, particularly in Queensland, it seems wise to respect the status of ‘endangered species’ conferred on *N. oxleyana* by the IUCN, Australian Society for Fish Biology, and Australian Commonwealth and NSW State governments, and to upgrade the status of *N. oxleyana* from ‘vulnerable’ to ‘endangered’ under the Queensland Nature Conservation Act 1992. Close monitoring is also required of the individual populations found in relatively well-protected habitats and aquatic systems (e.g. National Parks and World Heritage Areas) and particular emphasis should be placed on conserving the populations found in less well-protected areas of ‘wallum’ and other types of habitat supporting this endangered species. In Queensland, two other fish species, the ‘vulnerable’ *Pseudomugil melli* (Pseudomugilidae) and the ‘restricted’ *Rhadinocentrus ornatus* (Melanotaeniidae), and a range of aquatic invertebrates, especially insects (Chironomidae, Trichoptera, Odonata), are also restricted primarily to dystrophic waters draining wallum heathlands (Arthington and Watson, 1982; Arthington et al., 1986; Page et al., 2004). This concentration of species with specialised physiological and ecological affinities in dystrophic water bodies adds weight to our argument that these unusual aquatic systems
warrant conservation across the present geographic range of *N. oxleyana* in Queensland and NSW.

The distinctive relationships of *N. oxleyana* with features of aquatic habitat at the macro-, meso- and microhabitat scale demonstrate principles applicable to any study focused on the conservation of an endangered fish species. The first principle is the need to document the present-day distribution and threatening processes and, as a corollary, to collate evidence of ‘the ghost of disturbance past’. This would provide evidence to distinguish biogeographic patterns from the effects of human pressures, as well as an appreciation of the spatial scales at which the various threatening anthropogenic activities may operate and interact. Secondly, there is a need to understand meso- and microhabitat associations – the types of water bodies inhabited, their connectivity levels, if any, and their water quality and habitat characteristics. Connectivity potential is relevant for all water body types because hydrological connectance facilitates fish movements and dispersal at various spatial scales throughout the life cycle of the species. However, patterns of connectivity may require special attention in floodplain river systems where barriers, discharge regulation by dams/weirs, or human activities in the catchment (e.g. construction of levee banks) can disrupt the spatial patterns, timing, frequency, duration and extent of hydrological and biological connectivity (e.g. Poff *et al*., 1997; Bunn and Arthington, 2002; Arthington *et al*., 2005). Finally, there is a need to support macro-, meso- and microhabitat studies with a sound understanding of the drivers and processes that create and maintain habitat structure and connectivity within the broader landscapes of the overall distribution of the species (see Fausch *et al*., 2002; Stewart-Koster *et al*., 2007). Important environmental drivers include the natural river flow and sediment regime, riparian vegetation cover and condition, nutrient dynamics and other water quality features, and scale-related aquatic habitat structure (physical and biological). These environmental drivers and related ecological processes must be understood and managed at the appropriate spatial and temporal scales to support conservation actions for endangered fishes.

**ACKNOWLEDGEMENTS**

We wish to thank the agencies who supported these studies over the years, in particular the NSW Department of Primary Industries (DPI), Australian National Parks and Wildlife Service, Australian Nature Conservation Agency, Australian Research Council, Natural Heritage Trust, Griffith University, Southern Cross University, and our many colleagues who provided data or assisted with surveys in Queensland and NSW. Special thanks are due to Dr Fiona McKenzie-Smith and Dr Lyndon Brooks for assistance with the analysis of trapping
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data from Spitfire Creek and Evans Head, respectively. Permits for collection of *N. oxleyana*
and sampling within National Park estate in NSW were obtained from NSW DPI and NSW
Department of Environment and Conservation, respectively. Queensland fish surveys were
undertaken under the Griffith University fishing permit issued by the Queensland Department
of Primary Industries, and permits issued by Queensland National Parks and Wildlife.

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4: Conservation implications of distinct genetic structuring in an endangered freshwater perciform, *Nannoperca oxleyana* (Percichthyidae)

**Preface**

This chapter has been submitted for publication in a peer-reviewed journal.

Prof. P. Baverstock, C. Nock and M. Elphinstone were co-authors of the paper.

**Contribution to the preparation of this chapter:**

**Chapter Concept:** I was responsible for the conception of the research.

**Experimental Design:** Prof. P. Baverstock and I contributed equally to the experimental design.

**Sample Collection:** I was responsible for all sample collection.

**Laboratory and Data Analysis:** M. Elphinstone and C. Nock undertook the laboratory and data analysis.

**Data Interpretation:** Prof. P. Baverstock, C. Nock and I contributed equally to data interpretation.

**Writing:** I was responsible for writing the abstract, introduction and discussion. C. Nock and I were responsible for writing 80% and 20%, respectively, of the methods and results.
Conservation implications of distinct genetic structuring in an endangered freshwater Perciform, *Nannoperca oxleyana* (Percichthyidae)

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Abstract

The maintenance of genetic diversity and gene flow in threatened species is a vital consideration for recovery programs. The endangered Oxleyan pygmy perch *Nannoperca oxleyana* has a fragmented distribution within coastal freshwater drainages of southern Queensland and northern New South Wales (NSW), Australia. In this study, mitochondrial DNA control region variation was used to assess genetic diversity and structure across the geographic range of this species. Haplotypic diversity was highest in a small NSW subcatchment south of Evans Head (*h* = 0.594) followed by in Marcus Creek in Queensland (*h* = 0.475). Distinct genetic differentiation was evident among Queensland localities and NSW subcatchments, implying restricted gene flow between coastal river systems. One of the nine haplotypes detected was distributed over 83.4% of the species’ range, suggesting historical connectivity among the now fragmented populations. These patterns were concordant with eustatic changes associated with the last glacial maximum. High barrier sand dunes may also act as barriers to gene flow and dispersal between adjacent NSW subcatchments. Conservation efforts should focus on the preservation of genetic diversity by maintaining as many genetically differentiated populations as possible. The relatively diverse populations inhabiting the South Evans Head subcatchment and Marcus Creek require special management consideration.

**Additional keywords:** mitochondrial DNA, population structure, genetic diversity, fragmentation, floodplain connectivity, wallum.
**Introduction**

The maintenance of genetic diversity and gene flow in species threatened with extinction is a vital consideration for species recovery programs. A reduction in genetic diversity and changes in the distribution of this diversity among populations through population declines, fragmentation and isolation, and inappropriate restocking activities, can negatively affect a species’ reproductive fitness, evolutionary flexibility, local adaptations and/or co-adapted gene complexes, thereby increasing the likelihood of population or species extinction (Cowx 2002; Frankham 2005). To mitigate this threat and promote population and species viability, recovery programs should utilise knowledge of genetic diversity, population structure and gene flow to identify appropriate management units and areas of importance in maintaining dispersal, prioritise actions for conserving unique, source and declining populations, and determine the need for and appropriateness of restocking programs (Frankham *et al.* 2002; Allendorf and Luikart 2007).

The Oxleyan pygmy perch, *Nannoperca oxleyana* Whitley, is a small-bodied Australian percichthyid fish. The species is listed as endangered by the IUCN, and under Australian Commonwealth and New South Wales (NSW) state legislation. It is also listed as vulnerable under Queensland state legislation (IUCN 2004; NSW DPI 2005). Recovery plans have been developed for *N. oxleyana* (Arthington 1996; NSW DPI 2005), which aim to reverse declines in distribution and abundance attributed to habitat destruction, fragmentation and degradation, and introduced species (Pusey *et al.* 2004; Knight and Arthington 2008).

*Nannoperca oxleyana* is a habitat specialist, occupying swampy areas of dystrophic, freshwater systems draining the sandy, lowland, wallum (*Banksia*-dominated heath; see Griffith *et al.* 2003) ecosystems of coastal, mid-eastern Australia (Pusey *et al.* 2004; Knight and Arthington 2008). Populations are distributed along approximately 530 kilometres of coastline from Fraser Island in southern Queensland to Wooli in northern NSW (Figure 1). This distribution is currently fragmented into two main areas separated by several hundred kilometres of coastline, with coastal dispersal between and within these areas thought to be restricted by a salinity tolerance of less than 13.5 g. L$^{-1}$ (J. Knight, unpublished data). However, ‘chance’ movements via freshwater flood plumes are conceivable (Grimes and Kingsford 1996; Jerry and Baverstock 1998).
Figure 1. Map of coastal, mid-eastern Australia depicting the distribution of *Nannoperca oxleyana* (all squares), the sites sampled in this study (black squares) and by Hughes *et al.* (1999) (grey squares), the mtDNA haplotype frequencies (pie diagrams) at each Queensland site, and the three haplotypes in New South Wales (NSW) (see Figs. 2 and 3 for distribution of NSW haplotype frequencies). Data for populations from Queensland (excluding Little Canalpin Creek, North Stradbroke Island) were provided by J. Hughes, Griffith University, Queensland.
Within Queensland, *N. oxleyana* is known to inhabit 35 water bodies within 21 discrete drainage systems (Knight and Arthington 2008). Small populations inhabit six isolated, mainland drainages, while the remainder are distributed across Fraser, Moreton and North Stradbroke islands. Genetic analysis (based on mitochondrial DNA and allozyme data) of nine Queensland mainland and insular populations has revealed high levels of genetic structuring, inferring that they diverged from each other as a result of extremely limited dispersal (Hughes *et al.* 1999). There was, however, evidence for historical gene flow and subsequent divergence among Queensland mainland populations concordant with sea level rise following the last glacial maxima 8 000 – 10 000 years BP. Contemporary gene flow among interconnected swampy seepages and lakes on Moreton Island was also evident.

Since the work of Hughes *et al.* (1999), numerous additional populations have been discovered within the species’ southern distribution in NSW. Here, *N. oxleyana* has been found in 70 water bodies within 54 discrete systems draining the coastal subcatchments of the Richmond and Clarence rivers (Figure 1) (Knight and Arthington 2008). Approximately 80% of these water bodies occur in close proximity to one another on contiguous lowland, coastal floodplains. Populations also inhabit several adjacent subcatchments isolated from these floodplains by high (≤ 70 m) barrier sand dunes. The prevalence of *N. oxleyana* on the coastal floodplains may be attributed to intermittent connection among water bodies during high rainfall events or large floods emanating from the Richmond and Clarence rivers. These conditions may facilitate dispersal, thereby allowing the species to colonise new systems and/or to recolonise previously disturbed areas (Knight 2000; Knight and Arthington 2008).

Given the limited distributional range of *N. oxleyana* along the mid-eastern Australian coast it is reasonable to expect a similar phylogeographic pattern in NSW to that in Queensland proposed by Hughes *et al.* (1999). Thus, it is predicted that eustatic changes associated with the last glacial period may have facilitated wide-spread connectivity followed by isolation and genetic structuring among populations spread across the species’ distribution including in NSW. Likewise, the high sand dunes may also act as barriers to gene flow and dispersal between adjacent NSW subcatchments. However, as was found for the interconnected drainages on Moreton Island, we propose that contemporary gene flow may readily occur among intermittently connected water bodies within subcatchments on the NSW coastal floodplains. If the latter were correct, we would expect relatively high levels of genetic variation within and low levels of differentiation among intermittently connected subpopulations due to gene flow mediating natural divergence caused by genetic drift and/or
Genetic structuring in *Nannoperca oxleyana*

selection (Slatkin 1981; Frankham *et al.* 2002). Hence, we hypothesise that population genetic variation in NSW will follow a hierarchical spatial pattern (Meffe and Vrijenhoek 1988) wherein subpopulations within subcatchments exhibit less genetic divergence than populations from different subcatchments.

In this study we obtained information from mitochondrial control region variation to assess the genetic diversity and structure of *N. oxleyana* from previously unsampled sites in north-eastern NSW. Sequence data from Hughes *et al.* (1999) and from a population on North Stradbroke Island in south-eastern Queensland were also included in the study to provide a comprehensive overview for the species throughout its entire geographic range. We aimed to test the historical connectivity and hierarchical spatial pattern hypotheses. These analyses should inform decisions regarding the conservation management of this endangered freshwater fish.

**Materials and Methods**

*Sample collection*

In NSW, tissue samples were primarily pelvic fin clips taken non-destructively from 403 individuals from 39 water bodies between 2000 and 2004 during distributional surveys outlined in Knight and Arthington (2008). Sample sizes were typically small (mean ± S.E and range: 10.3 ± 2.3 and 1-72 samples per water body) because the species rarely occurs in large numbers (Knight 2000, in press). Supplementary samples were collected from the pelvic fins of 35 first generation captive-reared fish originating from Little Canalpin Creek on North Stradbroke Island, Queensland. These fish were considered representative of the wild population as they were bred from broodstock collected from multiple sites on multiple occasions as part of a conservation breeding programme (Knight *et al.* 2007). All fin clips were preserved in 70% ethanol until required.

Sampling in NSW was designed to test the floodplain dispersal and hierarchical spatial pattern propositions. Collections were made from all subcatchments known to support the species at present (Knight in press). Subcatchment boundaries were determined primarily on hydrologic grounds and based on the NSW classification scheme developed by NSW DLWC (1999). Special effort was made to sample locations that were geographically very close but physically and hydrologically separated by high sand dunes. Given the large number of sites sampled, the small sample sizes and the subsequent low haplotypic diversity found, subcatchments of the coastal, lowland floodplains of the Richmond and Clarence rivers that
lacked major geomorphologic boundaries were grouped to assist in data analysis and interpretation. This gave a total of five subcatchment areas including the ‘Richmond River coastal floodplain subcatchments’, which comprised the Coraki Area, Broadwater Area and Evans River subcatchments; an isolated ‘unclassified’ subcatchment of the Richmond River hereafter termed the ‘South Evans Head’ subcatchment; the ‘Clarence River coastal floodplain subcatchments’, which comprised the Esk River and Wooloweyah Lake subcatchments; and the Angourie/Redcliffe and Wooli River subcatchments of the Clarence River (Figures 2 and 3).

DNA extraction and amplification of control region sequences
Genomic DNA (gDNA) was extracted from each fin clip according to Elphinstone et al. (2003). The primers described by Hughes et al. (1999) (L19 and H16498) were used to initially amplify the hypervariable 5’ end of the mitochondrial control region in a few samples. Initial sequence data obtained for *N. oxleyana* enabled the design of a new species-specific primer (NaoxProL1, 5’ CAAAGCTAGGATTCTAAACTAACTATTCT 3’) located in the tRNA Proline gene flanking the control region which, along with H16498 (5’ CCTGAAGTAGGAACCAGATG 3’, Meyer et al., 1990), were used for all subsequent PCR amplification and sequencing.

All PCR amplifications were performed in 20-µL reaction volumes containing approximately 100 ng gDNA, 100 µM of each dNTP (Promega, www.promega.com), 1.5 mM MgCl₂, 100 nM of each primer, 2 µL of 10 x PCR reaction buffer (500 mM KCl, 100 mM Tris [Roche, www.roche.com]), 0.5 units of *Taq* DNA Polymerase and water to a final volume of 20 µL. PCR thermal cycles employed were: initial denaturation of 92°C for 1 min, followed by 30 cycles of 92°C for 10 s, 55°C for 30 s and 75°C for 1 min, and then a final extension at 75°C for 5 min. 5 µL of each PCR mixture was electrophoresed through a 1% agarose gel to confirm amplification.

Heteroduplex analysis/temperature gradient gel electrophoresis (TGGE-HA) analysis
Temperature gradient gel electrophoresis combined with heteroduplex analysis (TGGE-HA) was used to screen the amplified fragments for variation according to methods described by Elphinstone and Baverstock (1997). Perpendicular TGGE was applied to PCR product from a single individual from the Wooli subcatchment to optimise temperature gradient and run-time for parallel TGGE according to the manufacturer’s instructions (Qiagen GmbH,
www.qiagen.com). PCR product from this individual was then used as reference DNA for heteroduplex formation with all other DNA samples. Electrophoresis conditions for parallel TGGE were: the gel was 5% polyacrylamide:bis (37.5:1); runs were conducted at 300V for 140 minutes; and the temperature gradient used was 25 to 55°C. DNA was visualized by silver staining.

Where a distinct heteroduplex banding pattern was observed the individual was scored as a new haplotype. Where heteroduplex bands were not present that DNA sample was heteroduplexed with a second reference sample which the experimental procedure had already identified as different from the first reference sample; in this case the second reference sample used was from an individual from the Broadwater Area subcatchment.

**Sequencing**

DNA was sequenced directly using an ABI PRISM BigDye terminator cycle sequencing kit (version 3.1) and an ABI PRISM 3730 genetic analyser (Applied Biosystems, www.appliedbiosystems.com). Theoretically, TGGE can detect variants separated by a single base pair (Myers et al. 1987). The consistency of TGGE band patterns and scoring of gels was confirmed by sequence data collected in the forward and reverse direction from amplified mtDNA control region for at least two of each TGGE-HA NSW haplotypes and for samples from North Stradbroke Island, Queensland. Sequences were aligned using Chromas Pro version 1.41 (Technelysium Pty. Ltd., www.technelysium.com.au/chromas.html).

**Statistical analyses**

Haplotypic diversity and nucleotide diversity were calculated using ARLEQUIN version 3.0 (Excoffier et al. 2005). Mitochondrial DNA (mtDNA) sequences were analysed with a 95% probability cladogram estimation method using the program TCS version 1.21 (Clement et al. 2000) to determine the geographical and genealogical relationships of the haplotypes.

AMOVA as implemented in ARLEQUIN was used to assess the partitioning of genetic variation within and among populations (Excoffier et al. 1992). AMOVA calculates $\Phi$-statistics which are analogous to the $F$-statistics of Weir and Cockerham (1984). The Kimura 2-parameter model was selected using MODELTEST 3.7 (Posada and Crandall 1998). This model was considered appropriate as variation was distributed across the locus and consisted of only transitions. The $\Phi$-statistics were tested for significance using a permutation
Genetic structuring in *Nannoperca oxleyana*

To examine population dynamics, ARLEQUIN was also used to estimate Tajima’s D (Tajima 1989a, b) and to construct a mismatch distribution (Rogers and Harpending 1992). The mismatch distribution compares the frequency distribution of pair-wise sequence differences with that expected under sudden population expansion. A significant negative value of Tajima’s D indicates a population expansion, whereas a value significantly greater than zero represents a population bottleneck. For *N. oxleyana*, neither the generation time nor the mitochondrial control region mutation rate is precisely known. Recent data indicates that *N. oxleyana* mature at approximately 2 years and the maximum recorded age is 6.5 years (J. Knight, unpublished data). Using a minimum generation time of 2 years and an average mutation rate of 3.6% per million years for a range of fish species (Donaldson and Wilson 1999), the estimated time of population expansion (t, in generations) can be calculated using the formula $t = \tau/2u$ (Rogers and Harpending 1992), where $\tau$ is the mode of the mismatch distribution and $u$ is the mutation rate of the sequence ($u = 2\mu k$, where $\mu$ is the mutation rate per nucleotide and $k$ is the number of nucleotides).

**Results**

*Genetic Diversity*

Analysis of mtDNA variation was based on a 364-base pair fragment of which 12 nucleotides (3.3%) were polymorphic across all collection sites. In the NSW sample, two bases (0.55%) were polymorphic and three haplotypes were found (haplotypes A, H-I). Nucleotide diversity ($\pi$) and haplotypic diversity ($h$) ranged between 0-0.594 and 0-0.002, respectively, with most sites fixed for a single haplotype (Table 1). When sites were grouped by river subcatchments, a single shared haplotype (haplotype H) was found in each of the Richmond and Clarence coastal floodplain subcatchments (Figures 2 and 3; Table 1). Further south, the Angourie/Redcliffe and Wooli subcatchments were also fixed for a single haplotype (haplotype A). Haplotypic diversity was highest in the South Evans Head subcatchment ($h = 0.594$) (Table 1). All three NSW haplotypes were present in this subcatchment including one, the rare haplotype I (frequency = 0.083), found only in this subcatchment (Figure 2; Table 1).
Table 1. Population summary statistics for *Nannoperca oxleyana*: sample size, number of haplotypes, haplotypic ($h \pm$ S.E.) and nucleotide ($\pi \pm$ S.E.) diversities. Sequence data for populations from Queensland (excluding North Stradbroke Island) were provided by J. Hughes, Griffith University, Nathan, Queensland. NSW = New South Wales.

<table>
<thead>
<tr>
<th>Subcatchment or locality</th>
<th>No. of sites</th>
<th>Sample size</th>
<th>No. of haplotypes</th>
<th>$h$</th>
<th>$\pi$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NSW subcatchments</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richmond River Coastal Floodplain</td>
<td>13</td>
<td>114</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>South Evans Head</td>
<td>5</td>
<td>24</td>
<td>3</td>
<td>0.594±0.054</td>
<td>0.002±0.002</td>
</tr>
<tr>
<td>Clarence River Coastal Floodplain</td>
<td>16</td>
<td>217</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Angourie/Redcliffe</td>
<td>1</td>
<td>12</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Wooli River</td>
<td>4</td>
<td>36</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Queensland localities</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraser Island</td>
<td>1</td>
<td>18</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Moreton Island</td>
<td>3</td>
<td>93</td>
<td>3</td>
<td>0.084±0.039</td>
<td>0.0002±0.0005</td>
</tr>
<tr>
<td>North Stradbroke Island</td>
<td>1</td>
<td>35</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Searys Creek</td>
<td>1</td>
<td>13</td>
<td>2</td>
<td>0.154±0.126</td>
<td>0.0004±0.0070</td>
</tr>
<tr>
<td>Coondoo Creek</td>
<td>1</td>
<td>21</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Noosa River</td>
<td>1</td>
<td>32</td>
<td>2</td>
<td>0.062±0.058</td>
<td>0.0005±0.0080</td>
</tr>
<tr>
<td>Marcus Creek</td>
<td>1</td>
<td>28</td>
<td>2</td>
<td>0.475±0.067</td>
<td>0.004±0.003</td>
</tr>
<tr>
<td>Mellum Creek</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>
Figure 2. Distribution of *Nannoperca oxleyana* (all squares) north of the Clarence River in New South Wales, sites sampled during this study (black squares), and the mtDNA haplotype frequencies (pie diagrams) at each site. Topography (shaded) and subcatchment boundaries (dashed lines) are depicted. RRCFS = Richmond River coastal floodplain subcatchments. SEHS = South Evans Head subcatchment. CRCFS = Clarence River coastal floodplain subcatchments. Sample sizes within the RRCFS and CRCFS are given next to each site. NB. The southern termini of the CRCFS is depicted in figure 3.
Figure 3. Distribution of *Nannoperca oxleyana* (all squares) south of the Clarence River in New South Wales, sites sampled during this study (black squares), and the mtDNA haplotype frequencies (pie diagrams) at each site. Topography (shaded) and subcatchment boundaries (dashed lines) are depicted. ARS = Angourie/Redcliffe subcatchment. CRCFS = Clarence River coastal floodplain subcatchments. WRS = Wooli River subcatchment.
Haplotype frequency data for Queensland and NSW samples are presented in Figures 1-3 and a minimum spanning network derived from a 95% probability cladogram of their genealogical relationships is depicted in Figure 4. Overall, nine haplotypes were detected in *N. oxleyana* (GenBank Accession numbers EU275769-EU275777): seven haplotypes previously reported by Hughes *et al.* (1999) (haplotypes A-G), six of which were unique to Queensland, and two haplotypes found only in NSW (haplotypes H-I), (Figure 1; Table 1). Haplotype A, central in the network, was connected to the other haplotypes by a maximum of four nucleotide differences, and was distributed across 83.4% of the species’ geographic range on the mainland from Searys Creek in Queensland to Tick Gate Swamp in NSW (Figures 1-4). It was also present on North Stradbrooke Island, Queensland (Figure 1). The Queensland haplotype C appears to have derived from the NSW haplotype I (Figure 4).

Figure 4. Minimum spanning network for *Nannoperca oxleyana* showing genealogical relationships between haplotypes A-I. = unsampled or extinct haplotype. Data for populations from Queensland (excluding Little Canalpin Creek, North Stradbrooke Island) were provided by J. Hughes, Griffith University, Queensland.
Population Structure

AMOVA analysis revealed strong and statistically significant population subdivision among NSW subcatchments and Queensland localities based on both raw haplotypic and percent sequence divergence data (Table 2). More than 85% of the variation detected was among all subcatchments/localities, among Queensland localities and among NSW subcatchments. To test whether the monomorphic and disproportionately large samples from both the Richmond and Clarence River coastal floodplain subcatchments may have inflated the AMOVA results, a re-analysis was undertaken using reduced sample sizes of 30 for each for these two subcatchments. Little inflation was found with the $F_{ST}$ and $\Phi_{ST}$ values remaining highly significant ($P < 0.001$).

Table 2. Analysis of molecular variance (AMOVA) for *Nannoperca oxleyana* among all New South Wales (NSW) subcatchments and Queensland localities (see Table 1 for groupings), among all Queensland localities, among all NSW subcatchments, and among sites in the South Evans Head subcatchment based on raw haplotype frequencies ($F_{ST}$) and percent sequence divergence ($\Phi_{ST}$). $P$ values are based on 2 000 randomisations. *** = $P < 0.001$. Sum of squares (SS), Variance components (V), percentage of variation (%).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f</th>
<th>Analysis based on raw haplotype frequencies</th>
<th>Analysis based on haplotype frequency and percent sequence divergence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SS Var.</td>
<td>SS Var.</td>
</tr>
<tr>
<td>Among all subcatchments/localities</td>
<td>12</td>
<td>190.000 0.352 0.922***</td>
<td>394.027 0.731 0.931***</td>
</tr>
<tr>
<td>Among Qld localities</td>
<td>7</td>
<td>71.193 0.356 0.876***</td>
<td>199.554 0.998 0.899***</td>
</tr>
<tr>
<td>Among NSW subcatchments</td>
<td>4</td>
<td>45.926 0.018 0.915***</td>
<td>46.785 0.019 0.907***</td>
</tr>
<tr>
<td>Among South Evans Head subcatchment sites</td>
<td>4</td>
<td>1.500 0.021 0.071</td>
<td>1.933 0.041 0.120</td>
</tr>
</tbody>
</table>

Geographically close populations in adjacent subcatchments in NSW separated by high sand dunes were often distinct genetically (e.g. Bullocky Creek and Target Creek, Figure 2; sites east of Wooleweyah Lagoon, Figure 3). No genetic divergence was found between the Richmond and Clarence River coastal floodplain subcatchments in northern NSW given that a single shared haplotype was detected across 331 individuals from 29 sites (Figures 2 and 3; Table 1). By contrast, three haplotypes (with two haplotypes at greater than 40%) were detected in the South Evans Head subcatchment from 24 individuals from 5 sites (Figure 2).
AMOVA analysis among sites within this subcatchment was not significant ($F_{ST} = 0.071; P = 0.315$), with most of the variation detected within sampling sites (92.9%) (Table 2).

Population dynamics

Tajima’s D test of selective neutrality was negative as expected under population expansion, but non significant ($D = -0.285; P = 0.453$). However, the model of population expansion could not be rejected as the observed mismatch distribution was not significantly different to the predicted distribution of a historically expanding population (Rogers 1995) using two indices of fit (sum of squares differences $SSD = 0.03$, $P = 0.22$; and index of raggedness, $r = 0.01$, $P = 0.18$) (Figure 5). Estimated tau ($\tau$) = 0.469 and the assumed generation time and mutation rate suggest a population expansion time of 17,895 years.

Figure 5. Mismatch distribution of observed values (columns) and the expected distribution (line) according to the population expansion model among haplotype sequences of *Nannoperca oxleyana.*
Discussion

Knowledge of the population genetic structure and diversity of threatened species is of considerable importance to researchers and managers striving to conserve the remaining populations and returning the species to a position of viability in nature. This information aids in identifying appropriate management units for conservation and in the development and prioritisation of recovery actions aimed at maintaining important habitats and dispersal pathways, and conserving or enhancing unique, source and declining populations (Frankham et al. 2002; Scribner et al. 2006). This study of an endangered fish species, the Oxleyan pygmy perch, *Nannoperca oxleyana*, has explored the genetic structure and diversity of the species as part of broader efforts to guide recovery actions for this species in Queensland and New South Wales (NSW), Australia. Here major findings and their implications for the conservation of *N. oxleyana* are discussed.

Genetic structure

As expected, distinct maternal genetic structuring in *N. oxleyana* was evident at different spatial scales throughout its distributional range. Contemporary populations in coastal and island catchments thus appear isolated and genetically distinct, however, shallow haplotypic divergence and the presence of a shared, ancestral haplotype across the majority of species’ geographic range point to recent historic connectivity among the now fragmented populations. The derivation of the Queensland haplotype C from the NSW haplotype I implies that the NSW haplotype was previously more widespread thereby providing further evidence for historical connectivity between coastal drainages. Indeed, opportunities for northward and southward gene flow may have existed as recently as 8 000 years BP, at the end of the last glacial period, when lower sea levels facilitated shared confluences between adjacent drainages including the Richmond and Clarence rivers, and those in south-eastern Queensland (Lambeck and Nakada 1990; Hughes et al. 1999; Page and Hughes 2007; Knight in press). The data here suggest a range expansion around 18 000 years BP consistent with lower sea levels during the last glacial maximum (Neal and Stock 1986). Given uncertainty about estimates of mitochondrial control region mutation rate and generation time, any estimate of population expansion time is necessarily an approximation.

The intraspecific genetic pattern observed across the species’ range is typical of freshwater fish where dispersal between adjacent drainages is dependent upon the connectivity of freshwater (Ward et al. 1994; Rahel 2007). Thus, the loss of these dispersal pathways by rising sea levels following the glacial period may have lead to population fragmentation and
the patterns of genetic structuring observed in this study. It is also plausible that the high barrier dunes separating the NSW subcatchments may act as physical barriers to the dispersal of *N. oxleyana*, resulting in restricted gene flow and the formation of geographically isolated populations.

Populations inhabiting drainage systems within the Richmond and Clarence River coastal floodplain subcatchments and the Wooli River subcatchment lacked any genetic variability in the region sequenced. Hence, we were unable to determine if contemporary gene flow occurred within each of these areas between discrete water bodies connected laterally during flood events. Polymorphism was, however, detected in the South Evans Head subcatchment. Most of the variation found here was within sampling sites, which is suggestive of contemporary gene flow and dispersal between water bodies in this subcatchment (Ward *et al.* 1994). However, this inference should be treated with caution given the small samples sizes from this location. Further analyses using more sensitive molecular techniques such as microsatellite markers may elucidate patterns of contemporary gene flow and dispersal within subcatchments.

The conclusions for the South Evans Head subcatchment and the proposition that *N. oxleyana* disperses across the coastal floodplains of northern NSW are supported by the occurrence of this species in artificial water bodies including farm dams, remnant sand mining dredge ponds and drainage canals that are intermittently inundated by flood waters (Knight 2000; Knight and Arthington 2008). Indeed, lateral movements by conspecifics across these floodplains have been observed during localised flooding events (J. Knight, pers. observations), and the dispersal abilities of *N. oxleyana* are well documented, at least within extensive drainage systems (Arthington 1996; Hughes *et al.* 1999; Knight 2000; Pusey *et al.* 2004). Fluctuating water levels are characteristic of the wallum water bodies of mid-eastern Australia (Timms 1986; Knight 2000; Pusey *et al.* 2004). It is reasonable to assume that in months or years with high rainfall, swollen wallum drainages and inundated floodplains would provide ample habitat and food resources for *N. oxleyana* (Welcomme 1985; Junk *et al.* 1989), particularly in vegetated meso- and microhabitats protected from the destructive force of the main flow (Knight and Arthington 2008). These conditions may allow subpopulations, enlarged via increased recruitment success (Knight *et al.* 2007), to migrate within drainages and across the floodplain to colonise permanent and ephemeral habitats (Knight 2000; Balcombe *et al.* 2005). Alternatively, as observed by Knight (2000) in the Richmond River coastal floodplain subcatchments, the distribution and abundance of *N. oxleyana* may be substantially reduced in
dry months or years, through the desiccation of ephemeral habitats. A similar scenario has been documented for the Balston’s pygmy perch *Nannatherina balstoni* Regan inhabiting the peat flats of south-western Australia (Morgan *et al*. 1995).

**Genetic diversity**

Populations previously examined in Queensland had intrapopulation diversities ranging up to 0.476 at Marcus Creek (Hughes *et al*. 1999). The south Evans Head population exhibited the highest level of haplotypic diversity detected in this species (*h* = 0.594). Huey *et al*. (2006) recorded mean mitochondrial diversities of 0.215 and 0.363, respectively, for populations of two non-threatened siluriform catfish, *Neosilurus hyrtlii* and *Porochilus argenteus*, from the Coopers Creek catchment in western Queensland. The authors attributed the overall low haplotypic diversity of these two species to genetic bottlenecks brought about by drought-induced crashes in population sizes and concomitant losses in genetic variability (see also Douglas *et al*. 2003). This scenario of population booms and busts linked to drought-refuge source habitats and ephemeral sink habitats aligns closely to that of the *r*-selected life history pattern displayed by *N. oxleyana* on the coastal NSW floodplains (Knight 2000; Knight *et al*. 2007) and may account for the lack of genetic variation in mtDNA in these areas. Thus, the effects of drought may have eroded the genetic evidence for gene flow and dispersal expected to occur during flooding. In contrast, the South Evans Head subcatchment is dominated by large, deep dune lakes and permanent swampy drainage systems which presumably provide conditions conducive to the retention of genetic diversity.

Human activities have degraded the world’s biodiversity, caused many species to become extinct and placed many more in imperil (Groom 2006). In Australia, anthropogenic impacts are believed to have contributed to the contemporary presence/absence patterns of many freshwater fish species including *N. oxleyana* (Pusey *et al*. 2004; Knight and Arthington 2008), and may have influenced the observed genetic patterns in this study. Prior to their gazettal as National Park Estate in the late 20th century, the wallum heathlands in the NSW and Queensland study areas were subjected to high levels of disturbance via sand mining, forestry and logging, draining of wetlands, agriculture and urban/resort development (Wright 1991; NSW NPWS 1997; Pusey *et al*. 2004). The South Evans Head subcatchment escaped these disturbances through inclusion in a Commonwealth military training area since 1940. It is possible that these disturbances may have had a similar effect to that of drought on the genetic diversity of the previously unprotected *N. oxleyana* populations.
Conservation implications

Given the low level of genetic variation in this species and the vulnerability of physically and genetically isolated populations, it seems prudent to maintain the ‘endangered species’ status of *N. oxleyana*. This status will assist in concentrating efforts to protect and conserve the remaining macrohabitats, dispersal pathways and drought refuge habitats of *N. oxleyana*. The maintenance or restoration of connectivity may require special attention on the coastal floodplains where barriers such as roads and levee banks in the catchment can disrupt the spatial patterns, timing, frequency, duration and extent of hydrological and biological connectivity (Knight and Arthington 2008). Special management consideration should also be given to conserving the relatively diverse populations inhabiting the South Evans Head subcatchment in NSW and Marcus Creek in Queensland.

The distinct genetic structuring observed in this study indicates that fish from different Qld localities and NSW subcatchment areas have been isolated for a considerable period of time. As noted by Hughes *et al.* (1999) for Queensland populations, this should be taken into account throughout the species’ range. Future efforts to increase genetic diversity may be warranted for remnant populations suffering the effects of small population size. In such cases, the introduction of individuals from other populations can increase genetic variation and fitness affecting a ‘genetic rescue’ (Tallmon *et al.* 2004; but see also Hedrick 2005). However, predicting the outcome of such strategies in fish is problematic (McClelland and Naish 2007). In the absence of additional information from nuclear loci and comparative measurements of fitness in *N. oxleyana* populations, a precautionary measure would be to maintain as many genetically differentiated populations as possible and to avoid translocation between catchments.

Acknowledgements

This paper is from PhD research by J. Knight, supported by the Australian Research Council, NSW Department of Primary Industries (DPI) and Southern Cross University, Lismore. We thank J. Hughes for providing the Queensland haplotype sequence data and the numerous NSW DPI staff that assisted with sample collection. We also thank A. Arthington, D. Gilligan, J. Hughes, D. Jerry and an anonymous referee for comments on drafts of the manuscript. Sample collection was approved by the animal care and ethics committees of NSW DPI and Southern Cross University.
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Genetic structuring in *Nannoperca oxleyana*


Genetic structuring in *Nannoperca oxleyana*


5: REPRODUCTIVE BIOLOGY OF THE ENDANGERED OXLEYAN PYGMY PERCH

NANNOPERCA OXLEYANA WHITLEY

PREFACE

This chapter has been published in the peer-reviewed journal *Journal of Fish Biology*.


**Contribution to the preparation of this chapter:**

**Chapter Concept:** I was responsible for the conception of the research.

**Experimental Design:** I was responsible for the experimental design of the field- and aquaria-based research. P. Smith and Dr R. Wager provided detailed knowledge of animal husbandry and captive breeding techniques.

**Sample and Data Collection:** I was responsible for collection of all the aquarium-based data. G. Butler and I contributed equally to the collection of field-based biological samples and data. P. Smith provided the broodfish.

**Laboratory and Data Analysis:** I was responsible for all the laboratory and data analysis, except for the preparation of histological material, which was undertaken by staff at the Wollongbar Agricultural Institute.

**Data Interpretation:** I was responsible for 95% of data interpretation. P. Smith and Dr R. Wager provided 5% of data interpretation through the provision of some of the insights in courting behaviour.

**Writing:** I was solely responsible for the writing of this chapter.
Reproductive biology of the endangered Oxleyan pygmy perch

* Nannoperca oxleyana* Whitley

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(Received 15 August 2006, Accepted 18 June 2007)

The reproductive biology of the Oxleyan pygmy perch *Nannoperca oxleyana* Whitley is described from simultaneous studies of wild populations in north-eastern New South Wales and mature fish held in aquaria. In the wild, 50% of males and females matured at total lengths of 24.0-25.9 mm and 28.0-29.9 mm, respectively. The species displays sexual dichromatism during the spawning season, with males developing more intense red and brown fin and body colouration, and black pelvic fins. Captive male broodfish displayed territoriality during the breeding season, closely guarding sites within artificial, plant-like substrate in which pairs of fish spawned adhesive eggs. Protracted serial spawning of wild and captive fish occurred from September to April/May at mean water temperatures $\geq 16.6^\circ$ C and day length $\geq 10.7$ hours. Captive broodfish spawned on an average of 57% of days during the 256 day spawning period. Gonadosomatic indices averaged 0.7% for all ripe males and 4.1-4.2% for all ripe females collected. Mean total and batch fecundities of captive females were 1323 eggs/fish and 7.8 eggs/fish/day, respectively, and relative fecundity was 587 eggs/g of body weight. Batch fecundity of wild females was estimated at 7.8 eggs/fish. The adaptive significance of this reproductive strategy in a harsh, variable environment is discussed.

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Key words: *Nannoperca oxleyana*; reproduction; serial spawning behaviour.

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INTRODUCTION

The protection and conservation of fish species threatened with extinction is an important issue in today’s society. Species-specific programs aimed at preventing extinction involve, in part, utilising information on the species’ evolution, distribution, biology and ecology to mitigate human impacts and encourage population recovery (Carroll & Meffe, 1997; Burgman & Lindenmayer, 1998). In particular, an understanding of population fluctuations is integral to population conservation and requires insights into the links between demographic processes and the environments in which populations exist (Pulliam & Dunning, 1997). In turn, information on the reproductive biology of a fish species is essential for a comprehensive understanding of its population dynamics (King, 2003) and hence forms baseline data in the recovery planning process. This information is often lacking for many of Australia’s threatened fishes (Wager & Jackson, 1993; Knight & Butler, 2004; Pusey et al., 2004).

The Oxleyan pygmy perch, *Nannoperca oxleyana* Whitley is a small, freshwater, percichthyid fish endemic to the coastal wallum (*Banksia* dominated heath) ecosystems of northern New South Wales (NSW) and southern Queensland, Australia (Knight, 2000; Pusey et al., 2004). Degradation and loss of habitat associated with coastal development over the last 100 years has lead to declines in the species’ geographic range and size of populations. *Nannoperca oxleyana* is listed as an endangered species at an international, national and state level, and is the subject of a national recovery programme (NSW Department of Primary Industries, 2005). Currently a lack of biological information is a major impediment to the development of effective conservation and recovery actions.

Reproduction of *N. oxleyana* remains one of the least known aspects of its biology. Minimum total lengths (*L*T) of ripe fish collected in Queensland ranged from 22 to 27 mm for males and 22 to 30 mm for females (Pusey et al., 2004). In the Noosa River, reproduction was concentrated between October and December (Pusey et al., 2004). This period coincides with increasing day length and water temperature, and low probability of elevated discharge in south-eastern Queensland catchments. Anecdotal evidence suggests breeding may extend into late autumn at a lower intensity (Pusey et al., 2004). In aquaria, Wager (1992) observed daily serial spawning behaviour when water temperatures exceeded 20° C; pairs casually approached each other, shuddered while releasing a few eggs and milt, and then moved past one another. Females were capable of releasing up to 100 eggs over a 1 to 2 week period (Wager, 1992).
Despite these valuable insights, there is little understanding of the effort *N. oxleyana* invests in reproduction. Definitive information is lacking on important biological variables including total fecundity, the number of eggs released during a single spawning event (batch fecundity) and the number of spawnings in a season (spawning frequency). Published estimates of total fecundity (225 to 270 eggs per ripe female) and mean gonado-somatic indices ($I_G$) (ripe females: $3.3\%$, males: $0.6\%$) are based on only two females and 13 males collected from Moreton Island in Queensland towards the end of the breeding season in January 1995 when reproductive activity may have been reduced (Pusey *et al*., 2004; A. Arthington, pers. comm.). Fecundity estimates were also obtained from a single count of the standing stock of enlarged oocytes (A. Arthington, pers. comm.). Quantification of the reproductive effort of serial spawning fishes is difficult to gain with this method because such animals typically display indeterminate fecundity with new batches of eggs continually being recruited from small, immature oocytes throughout the spawning season. Total fecundity is best derived from summed estimates of batch fecundity, based on counts of hydrated oocytes and spawning frequency throughout the spawning season (Hunter & Macewicz, 1985; Hunter *et al*., 1985). These measurements, however, require intensive collections of large samples from wild populations and a strong understanding of the temporal processes occurring within the ovary (Hunter & Macewicz, 1985). Hence, accurate estimates of the effort invested in reproduction by *N. oxleyana* are difficult to obtain given the rarity and threatened status of the species.

In this paper, aspects of the reproductive biology of *N. oxleyana* are described from simultaneous studies of wild populations and captive broodfish. Temporal sampling of wild populations in north-eastern NSW provided data on the species’ $L_t$ at sexual maturity, reproductive cycle and mode and batch fecundity. Information on reproductive behaviour, spawning frequency and duration, total, batch and relative fecundity, the percentage of developing eggs and hatch rates was gathered by studying breeding pairs in aquaria.

**MATERIALS AND METHODS**

**STUDY SITES**

Three study sites were located near Evans Head, north-eastern NSW (29° 07’ S; 153° 26’ E) (Fig. 1). The Evans Head area receives an average annual rainfall of 1343 mm. Subtropical climatic conditions prevail with heavy periodic rains and humidity in summer and autumn, and moderate rainfall accompanied by high evaporation rates in late winter and spring. Average monthly temperatures range from 10° to 27° C over the year (Australian Bureau of Meteorology, 2005).
Reproductive biology of *Nannoperca oxleyana*

**Fig. 1.** Locations of study sites 1, 2 and 3 near Evans Head, north-eastern New South Wales, Australia.
Study sites were dystrophic, lentic systems with low nutrient status, low pH and low salinity, averaged 1.5 ha in size and 0.5 m in depth, and were surrounded by a riparian zone of *Melaleuca quinquenervia* and other wet heath species. The sedges, *Lepironia articulata* and *Philydrum lanuginosum*, and the aquatic moss *Sphagnum falcatum*, dominated the littoral zone and provided the majority of fish habitat.

FIELD SAMPLING AND PROCESSING OF FISH

Sites 1 and 2 were sampled initially in September 2003, January and March 2004 and then monthly between June 2004 and February 2005. Inadequate sample collections at these two sites following severe drought conditions in winter 2004 (Australian Bureau of Meteorology, 2005) required sampling at an additional site (site 3) monthly between February and May 2005. During the study, water temperatures at each site were recorded at 2 h intervals using Gemini Tinytalk™ TK-0014 data loggers [Gemini Data Loggers (UK) Ltd, Chichester, England, U.K.].

Fish were collected using collapsible, unbaited bait traps (240 x 240 x 400 mm, 3 mm mesh) set for 30 minutes. Each fish was killed in 100 mg l$^{-1}$ ethyl-p-amino benzoate (Barker *et al.*, 2002) and then measured ($L_T$ to the nearest 0.01 mm) and weighed ($M$, to the nearest 1 mg). The gonads were removed, examined under a stereomicroscope and assigned to a developmental stage (Tables I and II). Each gonad was weighed ($M_G$, to the nearest 0.1 mg). The gonado-somatic index ($I_G$) was calculated from: $I_G = 100M_G / M$. Data from each site were pooled for each month because of the low numbers of fish collected.

To assist in accurately staging development, all gonads were fixed in a solution of 10% formalin, 5% glacial acetic acid, 1% anhydrous calcium chloride and 84% seawater (FAACC), embedded in paraffin wax, sectioned at 5 µm, stained in Harris’ haematoxylin and eosin, and classified following the histological criteria listed in Tables I and II. Histological staging (see Fig. 2) was based on the most advanced oocytes in ovaries and the predominant cell types in testes (West, 1990; Scott & Pankhurst, 1992).

A total of 55 males and 63 females collected during the 2003-2004 and 2004-2005 breeding seasons (*i.e.* September 2003 to March 2004 and October 2004 to April 2005) were used to calculate size at sexual maturity, which was defined as the $L_T$ at which 50% of males had a moderate to high proliferation of spermatozoa or evidence of past reproductive activity ($\geq$stage 2b) and 50% of females had vitellogenic oocytes or regressing ovaries ($\geq$stage 3) (Marshall *et al.*, 1993; King, 2003).
Table I. Male macroscopic and histological staging criteria used for *Nannoperca oxleyana*. Macroscopic criteria adapted from Pollard (1972) and histological criteria from Scott & Pankhurst (1992), Gross *et al.* (2002) and Smith & Walker (2004) (see Fig. 2 for illustrated examples of germ cell and lobule development)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Category</th>
<th>Macroscopic testes</th>
<th>Microscopic Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Virgin/maturing</td>
<td>Gonads thin and threadlike, translucent, colourless and difficult to sex/ slightly rounded, translucent and greyish white to pale white.</td>
<td>Spermatogonia and primary spermatocytes predominate.</td>
</tr>
<tr>
<td></td>
<td>virgin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>a) Developing</td>
<td>Testes thickening, opaque, greyish white.</td>
<td>Small lobules containing spermatogonia and cysts of young cells (primary and secondary spermatocytes and spermatids). Rare spermatozoa may be present in lobule lumen.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Similar to 2a, but with additional elongated lobules containing diffuse residual spermatozoa.</td>
</tr>
<tr>
<td></td>
<td>b) Recovering</td>
<td>Testes large and rounded, greyish white often with clear translucent margins.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>spent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Late developing</td>
<td>Testes enlarged, greyish white</td>
<td>Moderate proliferation of spermatozoa within lobule lumen frequently surrounded by cysts of young cells. Some lobules may be enlarged and fully spermiated.</td>
</tr>
<tr>
<td>4</td>
<td>Ripe/partially</td>
<td>Testes plump, often bulbous, ivory white in appearance.</td>
<td>Most lobules enlarged, fully spermated and contain sparse peripheral spermatogonia and primary spermatocytes. Partially spent testes characterised by partial spermatozoa release and recruitment of young cell clutches.</td>
</tr>
<tr>
<td></td>
<td>spent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Spent</td>
<td>Testes shrivelled, flaccid, translucent greyish white.</td>
<td>Most lobules empty or containing diffuse residual spermatozoa and increased connective tissue. No obvious recruitment of younger clutches of cells.</td>
</tr>
</tbody>
</table>
Table II. Female macroscopic and histological staging criteria used for *Nannoperca oxleyana*. Macroscopic criteria adapted from Pollard (1972) and histological criteria from West (1990) and Marshall *et al.* (1993) (see Fig. 2 for illustrated examples of oocyte stages)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Category</th>
<th>Macroscopic ovary</th>
<th>Microscopic histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Virgin/ maturing virgin</td>
<td>Gonads thin and threadlike, translucent, colourless and difficult to sex/ more rounded ovaries occupying &lt;10% of body cavity. Translucent, clear to light orange with no visible oocytes (x40).</td>
<td>Perinucleolar: oocytes with thick, homogenous cytoplasm around a light nucleus containing few to many peripheral nucleoli. Cortical alveoli may be rare.</td>
</tr>
<tr>
<td>2</td>
<td>Developing</td>
<td>Ovaries rounded and thickening, typically occupying c. 40-60% of body cavity. Translucent orange with opaque oocytes just visible with naked eye or at x10-40.</td>
<td>Cortical alveoli: oocytes with cortical alveoli and oil droplets obvious in cytoplasm, increasing in size and number with development. Zona radiata thickens, stains pink with haematoxylin and eosin.</td>
</tr>
<tr>
<td>3</td>
<td>Late developing</td>
<td>Ovaries enlarged and rounded, typically occupying c. 80-100% of body cavity. Translucent to opaque orange, with opaque oocytes visible to naked eye.</td>
<td>Early vitellogenesis: oocytes with acidophilic yolk granules appearing in cytoplasm surrounding the nucleus. Cortical alveoli gradually become displaced to the periphery.</td>
</tr>
<tr>
<td>4</td>
<td>Ripe</td>
<td>Ovaries swollen and lumpy, occupying c. 90-100% of, and often distending, body cavity. Opaque orange with large opaque and translucent oocytes clearly visible to naked eye.</td>
<td>Nuclear migration, yolk fusion, and (pre-) ovulation: oocytes with yolk granules evenly dispersed throughout cytoplasm. Coalescence of yolk granules to form uniform yolk plates. Migration of nucleus to periphery of cytoplasm. Postovulatory follicles may be present. Marked increase in oocyte size and thickness of zona radiata.</td>
</tr>
<tr>
<td>5</td>
<td>Regressing</td>
<td>Ovaries misshapen and irregular in appearance, with loose walls, typically reducing to fill c. 40% of body cavity. Some dark yellow/brown patches (atretic oocytes) and opaque oocytes visible to naked eye. Abdomen not distended but flabby.</td>
<td>Atresia: perinucleolar and/or cortical alveoli oocytes present. High frequency of atretic oocytes and/or deteriorated post-ovulatory follicles.</td>
</tr>
</tbody>
</table>
Ripe ovaries from 14 females collected between July 2004 and April 2005 were used to determine batch fecundity and oocyte development patterns. Each ovary was teased apart in water under a stereomicroscope. The maximum diameter of a random sample of 200 oocytes from each gonad was measured in a random orientation (West, 1990). Batch fecundities were
estimated by counting the total number of hydrated oocytes present (Hunter et al., 1985; West, 1990).

CAPTIVE BROODFISH

Two 240 l glass aquaria were each divided into three equal portions, and set-up with a gravel substratum, a recirculating system employing an external Eheim® canister filter and a Resun® CL650 aquarium chiller, and filled with water of similar physico-chemical composition to habitats occupied by N. oxleyana near Evans Head (unpubl. data). Water temperature and photoperiod were adjusted monthly, based on data sourced from the field-based temperature loggers and Geoscience Australia (www.ga.gov.au/geodesy/astro/).

First generation, captive-reared broodfish (2.5 years old) were obtained from a conservation breeding programme run by Consolidated Rutile Ltd on North Stradbroke Island, Queensland. In November 2003, one pair of broodfish was established in each of five tank portions. At the beginning of the 2004-2005 breeding season, female and male broodfish measured 44.4-57.1 mm and 1.50-2.93 g and 45.7-52.0 mm and 1.39-1.92 g, respectively.

Artificial spawning habitat consisted of c. 75 strands of 25 cm black acrylic wool tied together at the top to form a ‘mop’. In each tank portion, one mop was hung vertically in the water column and two mops were laid horizontally on the substratum. Fish were fed a daily diet of frozen bloodworms intermittently supplemented with live Artemia salina (L.) and mosquito Aedes vigilax (Skuse) larvae.

Between November 2003 and June 2005, the spawning mops were regularly searched for eggs. Non-developing or moribund eggs were counted and then discarded. Developing eggs were counted and quickly aged (following criteria developed by Knight & Trnski, in press) before being returned to the tanks to hatch in floating Petri dishes.

Spawning frequency was defined as the number of days that broodfish spawned, batch fecundity as the number of eggs spawned in a day, total fecundity as the number of eggs spawned over the spawning season, relative fecundity as total fecundity per gram of body mass, and the number of eggs developing and hatch rate as the percentage of developing and hatched eggs of total number of eggs spawned. Reproductive behaviour was described through visual observations and by analysing six separate 24 h video recordings of four pairs of broodfish.

After June 2005, additional insights into spawning behaviour were gathered by removing the tank dividers and allowing broodfish to mix. To assist in verifying the gonad staging criteria, two reproductively active males and two females were killed in December 2005 and
processed in the laboratory following the procedures used for wild fish. The oocyte development pattern of an additional female was also examined. The $I_G$ for all five specimens was calculated.

RESULTS

A total of 189 *N. oxleyana* (15.6-52.0 mm $L_T$) were collected from the three sites during the study.

SIZE AT MATURITY IN THE WILD

The smallest mature male and female collected were 22.7 and 19.9 mm $L_T$, respectively, and all fish >30 mm were mature (Fig. 3). Fifty per cent of males were mature at 24.0-25.9 mm $L_T$, whereas 50% of females were not mature until 28.0-29.9 mm. These estimates are considered tentative given the small sample sizes for most $L_T$ classes.

![Proportion of sexually mature males (■) and females (□) collected during the 2003-2004 and 2004-2005 spawning seasons. Sample sizes are given above bars.](image)

REPRODUCTIVE BEHAVIOUR IN AQUARIA

Outside the breeding season, sexual dimorphism was not apparent. Both sexes were similar in morphology and colour, having a light brown to olive body colour with clear fins. At the onset of the breeding season, body and fin colouration of both sexes intensified, with red and brown pigments becoming more obvious. The dorsal, caudal and anal fin colouration
darkened and sexually dichromatic changes became apparent. Males developed more intense red colours. In addition, the males’ pelvic fins became jet-black, contrasting with the transparent dark fins of the females. Ripe females had a larger vent and extended abdominal cavity, through which orange coloured ovaries were visually recognisable in captive broodfish.

Spawning behaviour first became evident with males establishing territories encompassing the spawning mops. Males would swim parallel to females and turn sideways with erect dorsal and anal fins. This behaviour was often interspersed with periods of active pursuit of females, during which the female often retreated to the top of the water column. From the top of the tank the female would make advances towards the male, but was often aggressively chased away. Occasionally, the male accepted the female into the mops. The pair briefly rolled (shuddered, shimmied) through the spawning material, then parted. The female withdrew to cover or was sometimes chased by the male. Examination of spawning mops and video footage immediately following this behaviour validated that eggs were spawned during a rolling event (see: [www.dpi.nsw.gov.au/videos/pygmy-perch](http://www.dpi.nsw.gov.au/videos/pygmy-perch)).

The majority of rolling events occurred over a 1-3 h period, between 0700 and 1000 hours and most eggs were spawned at this time. On average, pairs would roll up to 12-13 times in an hour for periods of 10-30 s at a time. Occasionally eggs were also found that had been spawned in the afternoon. Eggs were not released during every rolling event as evidenced by fewer eggs found than the number of rolling events observed. On occasion, one to two groups of eggs at slightly different developmental stages were apparent, but it was difficult to ascertain exactly how many batches were released in a day. Hence, spawning frequency was defined as the number of spawning days in the season.

When the broodfish from each tank were allowed to mix, males readily established breeding territories and defended them aggressively from other males. Males either tried to court with any females that swam near their territory or chased them away.

REPRODUCTIVE CYCLES AND ENVIRONMENTAL VARIABLES

Only one pair of captive broodfish reproduced during the 2003-2004 breeding season. Spawning commenced after the pair had been established for 1 month, and occurred on 10 to 12 days per month in December 2003 and February to April 2004 [Fig. 4(c)]. No eggs were found between mid December and early February. Mean monthly water temperatures and day lengths during the spawning period ranged from 25.6° C and 14.0 h in December to 20.8° C and 11.4 h in April [Fig. 4(a)].
FIG. 4. Monthly reproductive indices of *Nannoperca oxleyana* and environmental variables recorded between September 2003 and May 2005 including (a) mean ± range (♦) water temperatures, and mean day length (◊) at study sites, (b) mean – s.e. gonado-somatic indices (*I*<sub>G</sub>) for wild males (♦) and females (◊) and mean total lengths of samples (see Fig. 5 for sample sizes), and (c) mean + s.e. spawning frequency (+ S.E.) of captive broodfish.

In the following season, spawning occurred over a 256 day period between September 2004 and May 2005 [Fig. 4(c)]. Spawning commenced after mean monthly water temperature rose by 3.1° C to 16.6° C and mean monthly day length increased from 11.1 to 11.9 h [(Fig. 4(a)]. All five pairs spawned between November and April. Spawning frequency peaked in November, occurring on a mean ± s.e. of 24.8 ± 0.7 days per pair. Thereafter, mean spawning frequency fluctuated until April 2005, due to infrequent spawning by one pair and an overall reduction in spawning by all pairs in January. Mean day length and water temperature peaked in December and January, respectively at 14.0 h and 25.5° C, before declining to 11.4 h and 21.5° C in April and to 10.7 h and 17.2° C in May [Fig. 4(a)].
Wild females followed a similar reproductive cycle. While there were no distinct seasonal variations in mean monthly $I_G$ values, the highest values were recorded in September 2003, March 2004 and between October 2004 and March 2005 [Fig. 4(b)]. These values generally coincided with high proportions of ripe females (stage 4) in the samples [Fig. 5(b)] as determined through macroscopic and histological techniques. The $I_G$ values and the percentage of ripe females declined after March 2005 as the proportion of fish with regressing gonads (stage 5) increased. Low $I_G$ values were associated with a predominance of fish in earlier stages of gonadal development (stages 1-3) in July and August. Two ripe females captured in July 2004 and February 2005 with exceptionally high $I_G$ values (10.2 and 11.5%, respectively) inflated the means and S.E. in these months.

![Graph showing monthly macroscopic gonadal stages for (a) male and (b) female Nannoperca oxleyana sampled between September 2003 and May 2005.](image)

**Fig. 5.** Monthly macroscopic gonadal stages (see Tables I and II) for (a) male [1 ( ), 2a ( ), 2b ( ), 3 ( ), 4 ( ) and 5 ( )] and (b) female [1 ( ), 2 ( ), 3 ( ), 4 ( ) and 5 ( )] Nannoperca oxleyana sampled between September 2003 and May 2005. Sample sizes are given above bars. NS, no sampling.

Low monthly sample sizes and mean lengths of some samples made interpretation of trends in the male reproductive cycle difficult [Figs. 4(b) and 5(a)]. As for females, high $I_G$ values and high proportions of ripe males were typically recorded in spring, summer and early autumn. In the months of April and May 2005, ripe males continued to dominate the samples,
even when most females contained ovaries that were regressing. Small proportions of ripe, spent and recovering spent (stage 2b) males and ripe and regressing females were collected in July and August 2004. All males collected in June 2004, however, were either ripe or spent. A reduction in female $I_G$ values in January of both seasons concomitant with an increase in the proportion of recovering spent males in January 2005 possibly reflects a temporary reduction in spawning effort in this month. Small virgins and maturing virgins (stage 1) were consistently captured throughout the study period.

The mean ± s.e. $I_G$ for ripe males was 0.7 ± 0.1% (range: 0.1-2.2; $n = 38$) compared to a corresponding female value of 4.1 ± 0.3 (range: 1.7-11.5; $n = 38$). Similarly, the mean $I_G$ for five ripe captive broodfish euthanased in December 2005 was 0.7 for males and 4.2 for females. The gonads of the reproductively active broodfish were classified both histologically and macroscopically as ripe (stage 5) gonads thereby supporting the accuracy of the criteria developed to classify gonad developmental stages of wild fish (Tables I and II).

**REPRODUCTIVE MODE AND EFFORT**

During the 2004-2005 breeding season, the captive broodfish spawned on a mean ± s.e. of 145.6 ± 23.4 days (57%) (range: 58-191 days) during the 256 day spawning period. Batch fecundity ranged between 1 and 51 eggs per day but had a mean ± s.e. and mode of 7.8 ± 0.9 and 1-2 eggs per day, respectively. Mean ± s.e. total and relative fecundity were 1323.4 ± 285.7 eggs per female (range: 405-2045 eggs per female) and 575.9 ± 98.2 eggs g$^{-1}$ (range: 270-818 eggs g$^{-1}$), respectively. The mean ± s.e. percentage of eggs developing was 87.5 ± 3.6% (range: 73.3-92.5%), and mean ± s.e. hatch rate was 75.2 ± 3.4% (range: 61.7-80.4%).

The size-frequency distribution of oocytes in ovaries from one ripe, captive broodfish and 13 of the 14 ripe, wild females is given in Fig. 6. The largest oocytes measured were 0.90 mm in diameter and all oocytes ≥0.85 mm were hydrated. All pooled individuals displayed similar oocyte size distribution patterns, with the polymodal distribution indicative of asynchronous oocyte development. Asynchronous oocyte development was also obvious in the histological sections of developing and ripe ovaries (Fig. 2).
The only ripe wild female collected towards the end of April 2005 had an ovary with an absence of oocytes between 0.45 and 0.875 mm in diameter and the presence of 0.90 mm oocytes. Hence, this fish appeared to be nearing the end of its spawning season with a small number of hydrated oocytes (n = 5) yet to be either spawned or resorbed and no new batches of mature eggs developing.

Hydrated oocytes were found in eight of the 14 ovaries examined from wild fish. Batch fecundities of these fish were 1, 1, 2, 2, 5, 6, 8 and 37 eggs with a mean ± s.e. of 7.8 ± 4.3 eggs and mode of 1-2 eggs. No hydrated eggs were found in the captive broodfish examined. The largest batch of hydrated eggs came from a large (50 mm) fish captured in late July 2004, which also had one of the highest I_G value recorded in the study (10.2%).

DISCUSSION

Reproduction in captive N. oxleyana broodfish mirrored that in wild populations, and the relatively high percentage of developing eggs and hatch rates validated the use of captive fish in this study and in the conservation breeding program initially developed by Wager (1992) and Consolidated Rutile Ltd.

The minimum L_T of sexually mature males and females were less than those reported for wild fish collected from the Noosa River, Queensland (males ≥27 mm, females ≥30 mm), but were similar to those reported from Spitfire Creek on Moreton Island, Queensland (both sexes ≥22 mm) (Pusey et al., 2004). In addition, L_T at which 50% of fish were mature were similar to estimates by Wager (1992) (both sexes >25 mm).
During the breeding season, *N. oxleyana* displayed sexual dichromatism, with males showing more intense changes. This is a trait typical of many species of fish (Liley & Stacey, 1983). The darkening of the males’ dorsal, anal and pelvic fins and red colouration of the body and caudal fin have been reported previously (e.g. Kuiter *et al*., 1996), but females were observed in this study to also undertake a similar, albeit less intense, colour transformation. The most obvious feature for distinguishing the sexes was the jet-black pelvic fins of the breeding males, which contrasted with the transparent dark fins of females. This method for sexing was evaluated by examining the fins and gonads of wild fish and was found to be an accurate, rapid and non-destructive method of sexing maturing and adult fish during the breeding season.

As reported by Wager (1992), captive broodfish exhibited external fertilisation and were serial spawners. The casual breeding behaviours reported by Wager (1992), however, contrast with the territoriality and specific acts of courtship and mating displayed in the present study. This territorial behaviour may represent defence of oviposition sites and/or a simple form of parental care, guarding eggs and larvae from predation. Indeed, Wager (1992) noted the possible cannibalism of young and predation of egg and larval stages and/or a similar form of territoriality or basic parental care has been reported for other species of *Nannoperca* (Shipway, 1949; Mitchell, 1976; Kuiter *et al*., 1996; Olney, 1999).

Similarities in the diurnal timing and displays of breeding behaviour have been reported for the western pygmy perch *N. vittata* Castelnau and other small-bodied Australian freshwater fishes (Shipway, 1949; Semple, 1985, 1986, 1991). Small fishes are also often observed to spawn within aquatic vegetation (Pusey *et al*., 2004). Presumably, the captive broodfish used the spawning mops for breeding in a similar way that fish would use aquatic and submerged vegetation in the wild. *Nannoperca oxleyana* has been observed to spawn within submerged vegetation in aquaria (Leggett, 1990; Wager, 1992), and wild populations occupy dense macrophyte beds and submerged riparian vegetation throughout the year (Knight, 2000; Pusey *et al*., 2004). Newly hatched, preflexion larvae have also been captured in these habitats (Knight & Trnski, in press). Given the combination of breeding behaviours observed in this study, *N. oxleyana* may be classified as having the reproductive strategy of a ‘guarder, substratum chooser and plant tender’ after Balon (1975).

The timing and duration of the spawning season was similar for captive broodfish and wild fish in northern NSW. The season commenced in September, coinciding with increasing day length and water temperature, which are generally recognised as the most important triggers of gonadal development and breeding activity in fishes (Lam, 1983). By late April, there was a reduction in the numbers of captive broodfish spawning, a decrease in $I_G$ values, cessation
Reproductive biology of *Nannoperca oxleyana*

of mature oocyte development, a reduction in the number of ripe females, and an increase in spent females in the wild.

Continued, albeit reduced, spawning activity in aquaria and the capture of a few ripe, wild fish after April supports the suggestion of Pusey *et al.*, (2004) that the breeding season may extend into late Autumn and beyond at a lower intensity. Spawning in Queensland was thought to curtail as early as January, although spawning duration was difficult to determine due to the low densities of fish collected (A. Arthington, pers. comm.). In the current study, there was evidence from both captive and wild fish of a reduction in reproduction in January and then a return to levels recorded before January, suggesting that the species may have two peak spawning periods, one from September to December and a second between February and April.

Like the captive broodfish, the wild fish also appeared to be serial spawners. Although the prolonged elevation of $I_G$ values during the spawning season could reflect a lack of population synchrony in gonadal development, the asynchronous oocyte development in ripe ovaries provides strong evidence of serial spawning. This implies that *N. oxleyana* has indeterminate annual fecundity (deVlaming, 1983; Hunter *et al.*, 1985).

Serial spawning, which overcomes the trade-off between total egg production and the limited abdomen space available to accommodate hydrated eggs (Wootton, 1992), increases the annual reproductive output of *N. oxleyana*, resulting in the high relative fecundity estimates derived in this study. In contrast, another Australian percichthyid the Murray cod, *Maccullochella peelii peelii* (Mitchell) grows to in excess of 20 kg in mass and spawns between 6800 and 86 600 eggs once per season, but has a relative fecundity of only 3.2-7.6 eggs g$^{-1}$ (Rowland, 1998).

Given the similarities between wild and captive fish for the duration and timing of the spawning season, mean $I_G$ values for ripe fish, and mean batch fecundity, it is plausible that estimates of spawning frequency and total and relative fecundity may also be similar. These estimates, however, may be at the upper limit of the species’ reproductive abilities as the captive broodfish were much larger than the size of fish at first maturity (Wootton, 1992). The estimates are also based on fish in a stable environment with plentiful food and no predation or competition. Hence, more energy may have been available for reproductive investment and output than would naturally occur in the wild. Conversely, there may be more and/or stronger spawning cues in the wild resulting in greater reproductive effort.

The reproductive biology of *N. oxleyana* is generally characteristic of the genus. These species mature at a small size, display seasonal sexual dichromatism, and spawn repeatedly within aquatic vegetation as water temperatures increase following winter (Table III). They
also have protracted spawning seasons, although *N. oxleyana* appears to spawn for a longer period. The mean $I_G$ values for ripe male and female *N. oxleyana* recorded in this study were similar to those reported in Queensland (Pusey *et al.*, 2004). The mean monthly values recorded during the spawning season, however, were much less than for *Nannoperca australis* Günther and *N. vittata* (Table III). While egg size is relatively similar among these three species, comparisons of published estimates of batch fecundity reveal much lower values for *N. oxleyana* than for *N. australis*. Hence, it appears that *N. oxleyana* has a lower yearly but more protracted investment in reproduction. Factors such as seasonal, interannual, and habitat related variations in abiotic conditions, dietary resources and egg size may also contribute to differences in reproduction among species within the genus.


<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>N. oxleyana</em></th>
<th><em>N. australis</em></th>
<th><em>N. obscura</em></th>
<th><em>N. variegata</em></th>
<th><em>N. vittata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Life span (years)</td>
<td>5+</td>
<td>3-4</td>
<td>5+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. recorded</td>
<td>60</td>
<td>85</td>
<td>75</td>
<td>62</td>
<td>68</td>
</tr>
<tr>
<td>$LT$ at maturity (mm)</td>
<td>Male: 25</td>
<td>Male: 30</td>
<td>Male: 35</td>
<td></td>
<td>Male: 42</td>
</tr>
<tr>
<td></td>
<td>Female: 29</td>
<td>Female: 33</td>
<td>Female: 40</td>
<td></td>
<td>Female: 43</td>
</tr>
<tr>
<td>Spawning season</td>
<td>September</td>
<td>September</td>
<td>September</td>
<td>July to</td>
<td>July to</td>
</tr>
<tr>
<td></td>
<td>to May</td>
<td>to January</td>
<td>to October</td>
<td>November</td>
<td>November</td>
</tr>
<tr>
<td>Minimum spawning temperature</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>temperature ($^\circ$C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg size (mm)</td>
<td>1.0-1.1</td>
<td>1.2-1.4</td>
<td>1.1-1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch fecundity (eggs)</td>
<td>1-51</td>
<td>78-679</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted batch fecundity (eggs)</td>
<td>44 mm: 4.7</td>
<td>44 mm: 287.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>57 mm: 9.7</td>
<td>57 mm: 548.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak mean monthly $I_G$</td>
<td>Male: 1.4</td>
<td>Male: 8.0</td>
<td>2.9</td>
<td>Female: 5.1</td>
<td>Female: 11.5</td>
</tr>
<tr>
<td></td>
<td>Female: 5.1</td>
<td>Female: 11.5</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

$I_G$, gonado-somatic index.
Batch fecundities are based on range of total lengths ($LT$) of *N. oxleyana* aquarium broodfish and are mean values for *N. oxleyana* and predicted values for *N. australis*.

The protracted, serial spawning behaviour of the genus *Nannoperca* may reflect an evolutionary adaptation for survival in harsh, variable environments. In particular, the water bodies of the wallum ecosystems inhabited by *N. oxleyana* are renowned for their fluctuations...
Reproductive biology of *Nannoperca oxleyana* in physico-chemical characteristics and water levels (Bayly, 1964; Bensink & Burton, 1975; Lee-Manwar *et al*., 1980; Timms, 1982). The sustained production of numerous small batches of eggs would theoretically allow for reproductive success during favourable conditions, while the loss of a few clutches during harsh periods may not be critical because of the relative small investment per spawning (deVlaming, 1983).

The protracted spawning behaviour and high relative fecundity of *N. oxleyana* may account for the occurrence of several large populations surveyed in Queensland and NSW (Knight, 2000; Pusey *et al*., 2004). There is also documented evidence of temporal population fluctuations driven by stochastic environmental change (Knight, 2000; Pusey *et al*., 2004). Population declines during extreme conditions (*e.g.* prolonged droughts) may be a product not only of increased juvenile and adult mortality, but also a reduction in reproductive and recruitment success.

This paper is from PhD research undertaken by J. T. K., and supported by NSW Department of Primary Industries (DPI), Southern Cross University (SCU), and the Australian Research Council. Consolidated Rutile Ltd supplied the captive broodfish and, in conjunction with the NSW Fisheries Scientific Committee, funded the histological preparations undertaken by staff at the Wollongbar Agricultural Institute. We thank those DPI staff who assisted with data collection, including G. Holder, G. Housefield, B. McCartin and N. Reed. In addition, A. Jordan, T. Pankhurst and S. Rowland assisted with developing appropriate methodologies and histological interpretation. We also thank B. Creese, A. Arthington, D. Pollard and S. Rowland for comments on drafts of the manuscript. This research followed animal care and ethics protocol 3/14 approved by SCU.

**References**


Reproductive biology of *Nannoperca oxleyana*


6: EARLY DEVELOPMENT OF THE ENDANGERED OXLEYAN PYGMY PERCH, NANNOPERCA OXLEYANA WHITLEY

PREFACE
This chapter has been accepted for publication in the peer-reviewed journal Australian Zoologist.

Dr T. Trnski was a co-author of the paper.

Contribution to the preparation of this chapter:
Chapter Concept: I was responsible for the conception of the research.

Experimental Design: I was responsible for the experimental design.

Data Collection: I collected all data with the exception of the larval morphological, meristic and pigment data, which was collected by Dr Trnski.

Data Analysis and Interpretation: I was responsible for all data analysis and interpretation except for the interpretation of larval morphological, meristic and pigment data, which was undertaken by Dr Trnski.

Writing: I was responsible for writing 70% of this chapter. Dr Trnski contributed to writing 15% of the results and 15% of the discussion on larval morphology, meristics and pigmentation.
Early development of the endangered Oxleyan pygmy perch *Nannoperca oxleyana* Whitley (Percichthyidae)

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The developmental ontogeny and morphology of the eggs, larvae and early juveniles of the endangered *Nannoperca oxleyana* is described based on collections of preserved wild fish, and preserved and live captive specimens reared at 25 ± 1°C. Eggs are telolecithal, spherical, average 1.02 ± 0.004 (± S.E.) mm in diameter, have a smooth, slightly adhesive chorion without filaments, a translucent homogeneous yolk, display meroblastic cell division and follow the general pattern of teleost embryogenesis. Early, middle and late stages of embryonic development were completed on average at 16, 28 and 50 hours post fertilisation. The larvae have generalised perciform morphological development with no apparent larval specialisations. The body is moderately deep bodied and compressed laterally. Head spination is limited to the development of an opercular spine in postflexion larvae. Pigmentation is relatively heavy and uniform over the head and body. Large melanophores occur on the dorsal, lateral and ventral midlines. Squamation commenced in postflexion larvae from 7.5 mm preserved body length (BL) and was complete in all specimens greater than 10.3 mm. Live captive, translucent larvae become pale green within two days of hatching and then light brown during the preflexion and postflexion stages. Juveniles are typically light brown laterally, darker dorsally and have a silvery-white belly. Growth of live, captive larvae and juveniles up to 6-months post hatching was described by the equation: \( \log_{10} \text{live BL} = 0.6043 + 0.0042A \) (\( r^2 = 0.917, P<0.001, n = 314 \)). Ranges for body length and age (days post hatch) of live captive fish were: preflexion 2.80-6.70 mm, 0-40 days; flexion: 6.35-7.70 mm, 21-61 days; postflexion: 6.40-10.30 mm, 43-118 days; and juveniles: 9.60-20.60 mm, 70-188 days. Live captive larvae commenced exogenous feeding at five days post hatching. Comparisons of *N. oxleyana* early developmental ontogeny and morphology are made with related percichthyids and sympatric species and implications for the conservation of the species are discussed.

Key words: *Nannoperca*, embryology, larval development, ontogeny
Early development of *Nannoperca oxleyana*

**Introduction**

Pygmy perch are a group of small, freshwater fishes endemic to southern Australia. The three genera of pygmy perch *Edelia*, *Nannatherina* and *Nannoperca* are considered members of the family Percichthyidae (previously Nannopercidae) (Hoese *et al.* 2006). Recent phylogenetic analysis supports the placement of the two species of *Edelia* (*E. vittata* and *E. obscura*) within *Nannoperca* (Jerry *et al.* 2001), which we follow here (although see Hoese *et al.* 2006). *Nannoperca oxleyana* Whitley inhabits coastal northern New South Wales (NSW) and southern Queensland (Qld), and represents the northeastern distribution limit of these genera (Kuiter and Allen 1986; Kuiter *et al.* 1996). *Nannatherina balstoni* Regan and *Nannoperca vittata* (Castelnau) are restricted to southwestern Australia, while *Nannoperca variegata* Kuiter and Allen, *Nannoperca obscura* (Klunzinger) and *Nannoperca australis* Günther are endemic to the southeastern corner of the country. Cryptic speciation exists with *N. vittata* and *N. australis* each apparently comprised of two distinct, yet undescribed species (Hammer 2002; M. Hammer, University of Adelaide, South Australia, pers. comm.).

Pygmy perch are amongst Australia’s most threatened fauna with four of the six described species threatened with extinction. Many populations have been lost or are under threat from habitat degradation and negative interactions with exotic species. A number of management plans have been developed with the aim of assisting these species to recover to a position of viability in nature (Fisher 1993; Arthington 1996; Hammer 2002; NSW Department of Primary Industries 2005). Recovery planning requires knowledge of the biology of a species to mitigate human impacts. Detailed biological data are therefore vital prerequisites in planning and implementing effective conservation management programs.

Although a number of studies have examined various aspects of the biology of pygmy perch, for example, reproduction (Knight *et al.* 2007), diet (Pen *et al.* 1993), habitat use, growth, diet and reproduction (Pen and Potter 1991; Arthington and Marshall 1993; Humphries 1995; Morgan *et al.* 1995; Arthington 1996), breeding in captivity (Llewellyn 1974; Wager 1992; Briggs 1999), and population genetics (Hughes *et al.* 1999; Hammer 2001), there remains a lack of research into their early life history. Studies of the eggs and larvae of pygmy perch are restricted to incomplete descriptions for *N. vittata* (Shipway 1949; Morgan and Beatty 2000) and *N. australis* (Llewellyn 1974), and a more comprehensive analysis of the larval development and diet of *N. balstoni* (Gill and Morgan 1998). This is indicative of the paucity of literature on Australian percichthyids, with the larvae of only six of 15 species fully described (Gill and Morgan 1998; Leis and Trnski 2004; Trnski *et al.* 2005).
Early development of *Nannoperca oxleyana*

*Nannoperca oxleyana* is recognised as the most threatened pygmy perch and is classified as endangered by the International Union for Conservation of Nature and Natural Resources (IUCN) and under Australian Commonwealth and State legislation (NSW Department of Primary Industries 2005). This species obtains a length of approximately 60 mm, is a microphagous carnivore, and inhabits shallow, swampy regions of dystrophic, acidic streams, lakes and swamps within coastal ‘wallum’ (*Banksia* dominated heath) ecosystems (Kuiter *et al.* 1996; Knight 2000; Pusey *et al.* 2004; Knight and Arthington, 2008). Protracted serial spawning occurs during the warmer months of the year between September and May when mean day length and water temperature reach and exceed 10.7 hours and 16.6°C, respectively (Knight *et al.* 2007). Despite a lack of research into the species’ early life history, aquarium enthusiasts have reported eggs hatching in one to four days at temperatures greater than 20°C (Leggett and Merrick 1987; Wager 1992). Larvae commenced feeding one to two days post hatching (Wager 1992) and grew to a total length of 18 mm within 10 weeks (Leggett 1990).

This study describes the developmental ontogeny and morphology of the eggs, larvae and early juveniles of *N. oxleyana* based on collections of captive reared and wild specimens, and constitutes the first complete larval description for the genus. The study provides baseline information for research into the habitat preferences, environmental tolerances, recruitment processes and population dynamics of *N. oxleyana*, for establishing successful conservation breeding and restocking programs if required, and contributes to a greater overall understanding of the conservation biology of this species.

**Methods**

**Animal husbandry**

Eggs and larvae were reared at the NSW Department of Primary Industries’ Port Stephens Fisheries Centre between November 2004 and May 2005. Techniques for breeding *N. oxleyana* are outlined in Knight *et al.* (2007). Briefly, broodstock were sourced from North Stradbroke Island, Qld. Six breeding pairs were housed separately in a recirculating, temperature controlled aquaria system. Adults were fed frozen bloodworms, live *Artemia* and mosquito wrigglers and spawned within acrylic spawning mops. Water quality, photoperiod (12 h light: 12 h dark) and water temperature (25 ± 1°C) for rearing eggs and larvae described in this study were based on average conditions experienced by wild populations in northern NSW during the spring/summer spawning period (Knight *et al.* 2007).
Spawning mops were checked regularly for eggs. Fertilised eggs were removed, counted and quickly assigned a developmental stage with the aid of a compound microscope. Embryos from the same parental stock and stage of development were grouped (termed a ‘batch’) and returned to the breeding tanks to hatch in floating petri dishes. Upon hatching, larvae from all parental stock were pooled, transferred to a flow-through fibreglass tank system (2.7 x 1.2 x 0.21 m) and housed in individual 2 litre cylindrical PVC holding containers at a mean ± S.E. stocking density of 19.6 ± 1.15 larvae per container. Water was gravity fed into each container and drained out the bottom through plankton mesh (0.5 mm), facilitating the exchange of water and removal of waste. Approximately 150 mL of live ‘green water’, dominated by cladocerans, rotifers and copepods (mean density: 16 zooplankters/mL$^{-1}$), was added to each container daily. Juveniles were fed once daily with live newly hatched Artemia nauplii. On two brief occasions, larvae were also supplied with Artemia nauplii during periods of low natural zooplankton production.

**Observations, measurements, terminology and counts**

Embryonic development was documented by examining 60 aquaria-reared eggs sampled from 23 batches. Development was divided into three broad stages in eggs including ‘Early’ – fertilisation to closure of blastopore, ‘Middle’ – closure of blastopore to tail bud lifting off yolk, and ‘Late’ – tail bud lifting off yolk to time of hatching (Matarese and Sandknop 1984). Rate of embryonic development (hours post fertilisation) was calculated by systematically observing the timing of developmental events for a series of eggs from the same batch. Time of fertilisation of a batch was estimated by viewing video footage of spawning brood fish. Average time of each developmental event was rounded to the nearest half hour and expressed as hours post fertilisation (h). Eggs were discarded after one observation to avoid possible effects on development as a result of stress.

Between five and 10 larvae were collected daily for the first 14 days post hatching and then at 3-5 day intervals for observation and measurement. Where required, additional specimens were collected to assist in identifying the ranges of ages (days post hatching) and lengths at each developmental stage. Larval development was divided into three broad stages including ‘Preflexion’ – hatching to commencement of notochord flexion, ‘Flexion’ – commencement to completion of notochord flexion, and ‘Postflexion’ – completion of notochord flexion to completion of squamation (Neira et al. 1998). Given that 0-day old larvae from all parental stock were pooled, the exact number of batches contributing to the study is not known. A total of 314 live larvae and juvenile fish from 97-170 batches were used to describe growth rates, colour in life, and the ontogeny of swimming and feeding (Table 1). Specimens were then
preserved in 70% ethanol for subsequent description. Specimens preserved for 6 months shrunk an average of 0.23 ± 0.011 (± S.E. and throughout text) mm in body length (Range: 0.0–0.9 mm) or 3.08 ± 0.16% (Range: 0.0 – 12.2%). The equation for estimating the body length of live larval and juvenile fish (LBL) from preserved body length (BL) for fish of 2.75 to 15.3 mm BL is: \( \text{LBL} = 0.0782 + 1.0195 \text{BL} \) \( (r^2 = 0.998, P<0.001, n = 186) \).

**Table 1.** Number, age and body length of larvae and juveniles included in the study. Developmental terminology adapted from Neira et al. (1998) and Leis and Carson-Ewart (2000). LBL = live body length. BL = preserved body length.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Captive reared</th>
<th>Wild</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days post hatch</td>
<td>Live specimens</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>LBL (mm)</td>
</tr>
<tr>
<td>Preflexion</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Yolk-sac</td>
<td>1-12</td>
<td>78</td>
</tr>
<tr>
<td>Yolk absorbed</td>
<td>8-40</td>
<td>58</td>
</tr>
<tr>
<td>Flexion</td>
<td>21-61</td>
<td>17</td>
</tr>
<tr>
<td>Postflexion</td>
<td>43-118</td>
<td>50</td>
</tr>
<tr>
<td>Juvenile</td>
<td>70-188</td>
<td>86</td>
</tr>
</tbody>
</table>

Larval morphological and meristic descriptions were primarily based on 37 captive reared, preserved specimens from 30-36 batches (Table 1). Data were also included from seven wild specimens collected in November 2005 from Evans Head, northeastern NSW (29° 04’S. 153° 23’E) using Perspex Quatrefoil traps set for 16 hours with a yellow cyalume light stick (Gilligan and Schiller 2003; Table 1). Wild-caught larvae were identified as percichthyids using the characteristics outlined in Leis and Trnski (2004), particularly their moderately deep body, relatively long gut, continuous dorsal fin, and counts of fin rays and myomeres. Wild larvae and juveniles were confirmed as *N. oxleyana* by comparison with captive reared specimens and meristic data for the species. Adult meristic counts are: D VI-VIII, 7-9 A III, 7-9 P₁ 11-13 P₂ I,5 Vertebrae 11-12+15-17 = 27-28 (Whitley 1940; Kuiter and Allen 1986; Kuiter et al. 1996; and Allen et al. 2002 and supplemented by x-rays of wild specimens housed in the Australian Museum fish collection).

Larval morphological definitions, measurements, and abbreviations follow Neira et al. (1998) and Leis and Carson-Ewart (2000). Eggs, larvae and juveniles were examined and measured under a dissecting microscope at magnifications from 8-50x. Precision of the measurements varied with magnification but ranged from 0.02 to 0.1 mm. Where larval morphometric values are given as a percentage, they are as a proportion of preserved body length unless otherwise indicated. All pigment described is external unless specified. Illustrations were prepared with
Early development of *Nannoperca oxleyana*

Early Development

Embryonic Development

**Early Development**

Fertilised egg (0.0 h): Eggs average 1.02 ± 0.004 mm in diameter (Range: 0.98-1.10 mm), are transparent, spherical with a clear, smooth, chorion (0.006 mm thick) and lack filaments, but are demersal and slightly adhesive. The yolk is translucent, unpigmented, unsegmented, and homogeneous in texture and averages 0.87 ± 0.007 mm in diameter (Range: 0.80-0.95 mm). Between 1 and 39 yellow-coloured oil globules are located centrally above the yolk surface and vary considerably in size from 0.01-0.35 mm in diameter. Their low density causes the globules to orientate upwards. The perivitelline space is relatively narrow at the vegetal pole, varying from 0.02-0.09 mm in thickness. As in all teleosts, egg development is telolecithal and the cleavage pattern is meroblastic.

One cell stage – blastopore closure (1.0-16.0 h): An accumulation of cytoplasm in the animal pole gives rise to the first cell measuring 0.52 mm in diameter (Fig. 1a). Mitotic division (Fig. 1b-d) results in the formation of a blastoderm with a cellular appearance (Fig. 1e) that transforms to a granular and then uniform appearance (Fig. 1f) as the cells divide and reduce in size. Epiboly commences. The dome shaped blastocoeil forms in the centre and the blastoderm becomes thinner and expands over the yolk mass towards the vegetal pole (Fig. 1g). The oil globules congregate in the yolk plug (Fig. 1h) prior to blastopore closure (Fig. 1i).
Early development of *Nannoperca oxleyana*

**Figure 1.** Early developmental stages of *Nannoperca oxleyana* eggs. a) one cell; b) two cells; c) eight cells; d) 16 cells; e) early blastoderm; f) blastoderm with granular appearance disappearing; g) epiboly; h) early yolk plug; i) gastrulation ending and neurulation commencing (NG, neural groove). Scale: Mean egg diameter is 1.02mm.

**Middle Development**

Early embryo – tail bud (16.0-23.5 h): The neural groove begins to develop along the embryonic axis just prior to the blastopore closure (Fig. 1i). Somites become differentiated, the cephalic region develops and the optic vesicles become visible (Fig. 2a). The early embryo reaches 190-200° around the yolk. Head width averages 0.24 mm, and the body reduces in width from 0.29-0.15 mm as it deepens, thereby creating a prominent neural ridge. Pigmentation begins with stellate melanophores averaging 0.01 mm in size becoming visible
on the dorsal then ventral area of the yolk-sac at 0.02-0.12 mm intervals. Melanophores also appear in rows on the tail and trunk of the embryo stopping short of the optic vesicles.

**Figure 2.** Middle and late developmental stages of *Nannoperca oxleyana* eggs. a) early embryo; b) embryo just prior to tail lifting off yolk; c) cephalic region developing in early embryo (PB, pectoral bud; OV, optic vesicle; L, lens); d) late stage embryo 290° around the yolk (PS, pericardial space); e) late stage embryo 360° around the yolk (FF, fin fold); f) late embryo ready to hatch; g) protuberance in the chorion caused by hatching larva; h) head free of chorion; i) hatching almost complete. Unless given, the scale = mean egg diameter of 1.02mm.

Tail free (25.0 h): As the subcaudal fold forms, the tail bud begins to separate from the yolk. The embryo circles the yolk by approximately 260° and the myomeres are defined (Fig. 2b). The optic vesicles, which are not yet closed ventrally, contain a lens 0.05 mm in diameter (Fig. 2c). The oil globules begin to coalesce, the pectoral buds form as small bulges laterally and posterior of the cephalic region, (Fig. 2c), and the pericardial space is visible (Fig. 2d).
Late Development

Late embryo (28.0-50.0 h): As the embryo grows to encompass the yolk by 290°, approximately 25% of the body separates from the yolk and the notochord typically ends close to the oil globules (Fig. 2d). The otic vesicles become obvious. The circulatory system begins to function. The tail continues to separate from the yolk, the fin fold forms and the optic vesicles close ventrally (Fig. 2e). The embryo begins regular active body flexing and tail wriggling.

Prior to hatching, the embryo increases in length encompassing the yolk by at least 380° with the tail reaching past the optic capsule (Fig. 2f). The average measurements for head width, body width and body depth are 0.42 mm, 0.17 mm, and 0.15 mm, respectively. The body and yolk are heavily pigmented with small stellate melanophores. A row of large melanophores are also present on the dorsal and ventral midlines of the tail. Typically, one large and a number of smaller oil globules are centred at the anterior end of the yolk-sac.

Hatching (50.0h): Most embryos hatch within 30 minutes. Hatching begins with a slight protuberance in the chorion in the cephalic region of the embryo as the chorion becomes flaccid (Fig. 2g). Repeated flexing by the embryo enlarges the protuberance until the chorion finally bursts and the larva emerges usually head first (Fig. 2h and 2i). The oil globules coalesce into a single oil globule between one and three days post hatching.

Larval Development

General morphology (Table 2, Fig. 3a-d)

Larvae are elongate (body depth, BD 16-20%) at first hatch and until after the yolk is absorbed. The body becomes moderately deep in preflexion larvae from 5.0 mm BL and does not exceed 33% of BL. The body and head are moderately laterally compressed. There are 27-30 myomeres (9-14 preanal and 15-20 postanal); the anus is located under myomeres 9-10 until yolk-sac absorption, and under myomeres 11-14 in larger larvae. The gut is twisted in yolk-sac larvae and is coiled and triangular in preflexion larvae from 4.6 mm BL. The preanal length is 41-53% in preflexion larvae, and the gut becomes longer and elongate from the flexion stage (48-64%). The swim bladder is first visible in yolk-sac larvae from 4.2-4.3 mm BL; it is located over the midgut, always conspicuous and is usually large. The notochord tip is relatively elongate during notochord flexion. The round to slightly elongate head is initially small to moderate (head length, HL 17-22%), is moderate by the time yolk is fully absorbed and is large in postflexion larvae and juveniles (HL 35-40%). The snout is slightly convex, short and is never more than 50% of the eye diameter. The eye is round and large (eye
diameter, ED 36-49% HL). The small to moderate mouth reaches midway between the anterior margin of the eye and the pupil. Teeth are not apparent in any of the larvae or juveniles examined. The nasal pit forms by the flexion stage, and roofs over in juveniles, by 8.9-9.5 mm. Larval head spination is absent. A weak opercular spine is present from the flexion stage.

**Meristics (Table 3; Fig. 3a-d)**

Dorsal- and anal-fin anlagen form by the commencement of notochord flexion. Bases and incipient rays of the dorsal and anal fins develop during notochord flexion, and the elements are ossified in postflexion larvae from 6.3 mm BL. The full complement of dorsal and anal elements is present by 6.3-8.5 mm BL. Anal-fin counts in reared larvae may be outside published ranges; this appears to be an artifact of rearing. The pectoral fins begin to form incipient rays in postflexion larvae from 7.3 mm BL, the rays ossify from dorsal to ventral and all are present by 8.5 mm BL. Pelvic-fin buds appear in preflexion larvae from 5.4 mm BL, the membrane forms during flexion, and ossification of the elements commences in postflexion larvae from 6.3 mm BL. The pelvic-fin elements are all ossified by 7.6 mm BL. Caudal-fin anlage appear in preflexion larvae from 5.4 mm BL, and rays commence ossification in flexion larvae. All primary caudal-fin rays are ossified in postflexion larvae by 7.6 mm BL. The smallest and largest larvae undergoing notochord flexion were 6.0 mm and 7.2 mm BL.
Table 2. Morphometric data for preserved, captive reared and wild larvae and juveniles of *Nannoperca oxleyana*. Measurements are in mm. * indicates drawn specimen. + indicates photographed specimen.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Days post hatch</th>
<th>Total length</th>
<th>Body length</th>
<th>Preanal length</th>
<th>Pre dorsal fin length</th>
<th>Body depth</th>
<th>Head length</th>
<th>Snout length</th>
<th>Eye diameter</th>
<th>Vent to anal fin length</th>
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</thead>
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<td>3.05</td>
<td>1.35</td>
<td>0.62</td>
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<td>0.23</td>
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<td>4.15</td>
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<td>4.15</td>
<td>1.70</td>
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<td>0.87</td>
<td>0.10</td>
<td>0.35</td>
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<tr>
<td>Preflex</td>
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<td>4.32</td>
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<td>1.00</td>
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<td>Preflex</td>
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<td>1.21</td>
<td>0.22</td>
<td>0.50</td>
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Figure 3. Larvae of *Nannoperca oxleyana*. a) yolk-sac larva; b) preflexion larva; c) flexion larva; d) postflexion larva. Larval body length (mm) is given in each figure.
Early development of *Nannoperca oxleyana*

**Table 3.** Meristic data for preserved, captive reared and wild larvae and juveniles of *Nannoperca oxleyana*. Incipient elements are given in parentheses. Myomeres are divided into pre- and post-anal counts (Pre and Post, respectively). * indicates drawn specimens. + indicates photographed specimen.

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Squamation

Squamation does not begin until postflexion larvae attain 7.5 mm BL. A single row of clear, ctenoid scales initially appear along the notochord while the median fins are still developing. The scales spread along the body laterally, then dorsally and ventrally. The last regions to become scaled are the opercula and ventrally around the pelvic fins, followed by the nape, and finally the hypural bones. The smallest fully scaled juvenile measured 9.2 mm BL and all specimens greater than 10.3 mm had completed squamation.

Pigment (Fig. 3a-d)

Larvae are moderately pigmented at first hatch and become heavily pigmented by the time the yolk is absorbed. Melanophores are concentrated on the dorsal and ventral midlines of the tail, and midlateral surface of the head and trunk. Small expanded melanophores are present at the tip of the upper and lower jaws, ventrally along the lower jaw, dorsally on the head and over the operculum. The nasal pit is unpigmented. Internal melanophores are present along the roof of the mouth, and posterior to the eye below the mid- and hindbrain. A matching horizontal band of external melanophores is present on the operculum in line with the eye until the early postflexion stage. The eye is initially unpigmented, but becomes pigmented during yolk absorption (by 4.2 mm BL).

Small melanophores are present along the ventral midline of the gut, from anterior of the cleithral symphysis to the anus. Additional melanophores cover the remainder of the gut except there may be an unpigmented region immediately above the pelvic-fin base in preflexion and flexion larvae.

Five to seven large, expanded melanophores are present along the dorsal midline of the trunk (1 or 2 melanophores) and tail (4 or 5), from the nape to the caudal peduncle; these are apparent in preflexion larvae by the time the yolk is absorbed. A series of melanophores is present along the lateral midline of the trunk and tail, commencing at the swim bladder and extending to the posterior end of the dorsal and anal fins. In postflexion larvae, this series extends onto the anterior third of the caudal peduncle. Up to six expanded melanophores are present along the ventral midline of the tail, from above the anus to the caudal peduncle. Between one and three of these expanded melanophores occur along the anal-fin base. A small melanophore is occasionally present in early postflexion larvae at the base of ventral primary caudal-fin rays 1-2. Most of the expanded melanophores on the dorsal and ventral midlines migrate internally and become difficult to distinguish by the juvenile stage. Only two dark patches remain in juveniles, one each on the dorsal and ventral margins of the caudal
Early development of *Nannoperca oxleyana*

peduncle immediately posterior to the dorsal and anal fins, respectively. Internal melanophores are present over the swim bladder, the mid- and hindgut, and may be present along the notochord. The external and internal pigment series thus give the impression of a line of heavy pigment from the tip of the snout, across the head and trunk to the tail. A large black dot, characteristic of the species, begins to form at the base of the caudal fin as a tight group of small black melanophores in flexion larvae and is completely developed in postflexion larvae.

By the juvenile stage, additional melanophores develop laterally on the head and body, and all the fins become pigmented. Melanophore coverage is lightest ventrally on the head and gut. Three broad vertical bands become apparent dorsally on the nape, below the centre of the spinous dorsal fin and below the centre of the soft dorsal fin in juvenile fish from 13.5 mm in length.

*Colour in life*

At hatching, live larvae are transparent. With the exception of the yolk, larvae turn pale green within two days of hatching (Fig. 4a). A black line also forms ventrally along the tail, running above the yolk to the posterior of the eye. Pigmentation develops in the eyes and branching chromatophores appear, occurring dorsally and ventrally on the trunk and tail. At four days post hatching (3.9-4.3 mm LBL), the eyes become fully pigmented taking on a speckled black and gold appearance. The black line along the tail fades in preflexion larvae, remaining only above the swim bladder (Fig. 4b), and is absent in juveniles. The branching chromatophores also gradually dissipate until they are no longer visible in flexion larvae (Fig. 4c). The nape and dorsal area of the head of preflexion larvae become light brown, followed by the trunk and tail in flexion and postflexion larvae (Fig. 4c and d). Iridescent golden scales develop in postflexion larvae on the operculum, laterally on the stomach and ventrally around the pelvic fins. Fully scaled juveniles are typically light brown laterally, darker dorsally and have a silvery-white belly (Fig. 4e). The iridescent scales remain while all other scales have black margins with small peppery melanophores. Sparse red pigmentation also occurs in the skin on the trunk of some postflexion and juvenile fish.
Figure 4. Micrographs of live larvae and a juvenile of *Nannoperca oxleyana*. a) yolk-sac larva, 3 days post hatch, 4.0mm BL; b) late preflexion larva, 25 days post hatch, 6.0mm BL; c) late flexion larva, 52 days post hatch, 7.6mm BL; d) late postflexion larva, 56 days post hatch, 8.6mm; e) juvenile, 136 days post hatch, 12.9mm BL.
Early development of *Nannoperca oxleyana*

**Growth**

The growth of 314 live, captive reared fish over a 6-month period is depicted in figure 5. Initial growth was relatively rapid. Newly hatched larvae (2.8-3.4 mm LBL, mean: 3.17 ± 0.027 mm LBL), increased in mean length by 0.49 mm in the first 24 hours post hatching. Mean growth rates decreased over the following three days to 0.28 mm, 0.21 mm and 0.09 mm, respectively and then proceeded to plateau at a mean daily increase of only 0.02 mm in larvae 5-10 days post hatching. Growth rates returned to a mean daily increase of 0.23 mm in 14-day-old larvae (LBL: 4.56 ± 0.075 mm). Beyond this period, growth rates became more variable. The mean monthly LBL of fish of 1 to 6-months of age were 5.74 ± 0.140 mm, 8.78 ± 0.252 mm, 9.80 ± 0.401 mm, 11.46 ± 0.330 mm, 17.24 ± 0.259 mm and 18.65 ± 0.255 mm, respectively.

![Figure 5. Growth and development of captive reared *Nannoperca oxleyana* larvae and juveniles over 188 days (n = 314). Development includes preflexion ●, flexion △, postflexion ■, and juvenile ◊ stages.](image)

There was increased variation in length as fish aged, with the coefficient of variation ranging from 2 to 4% up to 17 days post hatching, to as high as 19% for older larvae. Variation in the distribution of residuals from the linear regression of LBL at age violated the assumption of homogeneous variances. This problem was best resolved by a log 10 transformation of body length (Log$_{10}$ LBL), with the residual plots showing no increase in variance with age (A). Hence, larvae and juveniles showed logarithmic growth for the 6-month period, which is best described by the equation: Log$_{10}$ LBL = 0.6043 + 0.0042A ($r^2 = 0.917$, P<0.001, n = 314).
Body length was a more precise measure of larval development than age as there was less overlap of the major developmental stages with body length than with age (Fig. 5; Table 1). Newly hatched larvae took up to 40 days to reach the end of the preflexion stage at a maximum LBL of 6.70 mm. The flexion stage was almost completely overlapped by the preflexion and postflexion stages in terms of body length and age. Flexion larvae ranged in size from 6.35-7.70 mm LBL and in age from 21-61 days post hatching. The smallest and youngest postflexion larvae were 6.40 mm LBL and 43 days old, respectively, and the largest and oldest were 10.30 mm LBL and 118 days old. However, the smallest juvenile studied measured 9.6 mm LBL and the youngest was 70 days post hatching.

Feeding and Swimming Ontogeny

Given that larval length remained relatively constant between the ages of 5 and 10 days post hatching, age was used to describe the ontogeny of larval feeding and swimming. The yolk sustained larvae for the first four days post hatching. During this time, the head separated from the yolk, the eyes became pigmented, the jaw developed, detached from the yolk and opened, and the gut formed in the place of the yolk-sac.

Newly hatched larvae were unable to swim or maintain buoyancy and typically remained motionless on the substrate. When disturbed, larvae briefly kicked sideways off the substrate in a spiralling motion then relaxed and sank. As the pectoral fins developed, larvae became more active, but still swam in a spiralling motion in a lateral orientation. Swim bladder inflation commenced 4 days post hatching, and 24 hours later all larvae were able to maintain buoyancy in the water column and swim freely in a dorsal-ventral orientation. At this time, larvae were 3.90-4.55 mm LBL and were often seen near the water surface presumably searching for food.

As the larvae developed, the yolk-sac was absorbed and gradually decreased in size (Fig. 6). Yolk-sac length decreased by a mean of 62% between 4 and 5 day old larvae. This coincided with increased development of the mouth and the commencement of exogenous feeding, as evidenced by peristalsis, the presence of green algae in the stomach and intestines, and waste material in the lower intestine and anus. Complete yolk-sac absorption was first observed in eight day old larvae, and had occurred in all larvae greater than 12 days of age (Fig. 6).
**Discussion**

The eggs of *N. oxleyana* are typical of those of most teleosts, being telolecithal and spherical, having a smooth chorion and translucent, homogeneous yolk, displaying meroblastic cell division and following the general pattern of embryogenesis (Blaxter 1988; Kendall *et al.* 1984). In addition, the eggs are demersal as in most freshwater fishes and percichthyids (Johnson 1984; Kendall *et al.* 1984; Harris and Rowland 1996; Pusey *et al.* 2004), and are morphologically similar to those described for other *Nannoperca* species (Shipway 1949; Llewellyn 1974; Morgan and Beatty 2000). A lack of filaments on the chorion is a further feature characteristic of Australian percichthyids (Harris and Rowland 1996; Pusey *et al.* 2004).

Within the Percichthyidae, the size of *N. oxleyana* eggs (0.98-1.10 mm) is similar or slightly smaller than those described for *N. australis* (1.16-1.35 mm), *N. vittata* (1.1-1.2 mm), the two catadromous *Macquaria* species (0.9-1.5 mm), and *Bostockia porosa* (1.4-1.8 mm), and is much smaller than those of *Maccullochella* spp. and the two freshwater *Macquaria* species (2.5-4.2 mm) (Shipway 1949; Lake 1967; Llewellyn 1974; Cadwallader and Rogan 1977; Pen and Potter 1990; Harris and Rowland 1996; Neira *et al.* 1998; Morgan and Beatty 2000; Pusey *et al.* 2004; Trnski *et al.* 2005).
Development of the eggs was very similar to that of *N. australis*, although the development rate was faster in *N. oxleyana* (i.e. early development 16 hrs vs 27 hrs, middle development 25 hrs vs 39.5 hrs, late development 50 hrs vs 74 hrs). The shorter embryonic period may have been at least in part a result of different rearing temperatures, with *N. australis* reared at lower temperatures ranging between 15.8 and 25.3º C (Llewellyn 1974). Eggs of *N. oxleyana* spawned and reared at 16º C took approximately 144 hrs to hatch (J. Knight, unpublished data).

Throughout its distributional range, *N. oxleyana* is known to co-occur with 16 species of fishes belonging to the families Eleotridae, Melanotaeniidae, Pseudomugilidae, Galaxiidae, Ambassidae, Plotosidae, Percichthyidae, Anguillidae, and Poeciliidae (Arthington and Marshall 1993; Arthington 1996; Knight 2000, in press). Of these, the two Anguillids spawn in the deep ocean (Allen *et al.* 2002), the poeciliid *Gambusia holbrooki* is viviparous (live bearers) (Milton and Arthington 1983), *Galaxias maculatus* spawns eggs that develop out of water on moist, riparian vegetation (Pollard 1971; Allen *et al.* 2002), and the percichthyid *Macquaria novemaculeata* is catadromous, breeding in estuarine areas in winter (Harris 1986; Trnski *et al.* 2005). Eggs of *N. oxleyana* can be distinguished from those of the remaining sympatric species through a combination of egg shape, size and the absence of filaments (Table 4).

### Table 4. Major distinguishing characteristics of the eggs of *Nannoperca oxleyana* and 11 sympatric indigenous species. Data sourced from Lake (1967); Anderson *et al.* (1971); Llewellyn (1971); Auty (1978); Howe (1987); Koehn and O’Connor (1990); Semple (1986, 1991); and Pusey *et al.* (2004).

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus/Species</th>
<th>Shape</th>
<th>Size (mm)</th>
<th>Filaments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambassidae</td>
<td><em>Ambassis agassizii</em></td>
<td>spherical</td>
<td>0.6-0.7</td>
<td>no</td>
</tr>
<tr>
<td>Eleotridae</td>
<td><em>Gobiomorphus australis</em></td>
<td>unknown</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td></td>
<td><em>Hypseleotris compressa</em></td>
<td>slightly pear shaped</td>
<td>0.26-0.28 x 0.30-0.32</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td><em>Hypseleotris galii</em></td>
<td>oblong</td>
<td>0.91 x 0.62</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td><em>Philypnodon grandiceps</em></td>
<td>elongate to elliptical</td>
<td>1.5-2.2 x 0.7-0.9</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td><em>Philypnodon macrostomus</em></td>
<td>teardrop-shaped</td>
<td>unknown</td>
<td>no</td>
</tr>
<tr>
<td>Melanotaeniidae</td>
<td><em>Melenotaenia duboulayi</em></td>
<td>spherical</td>
<td>0.88-1.5</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td><em>Rhadinocentrus ornatus</em></td>
<td>spherical</td>
<td>1.2-1.35</td>
<td>yes</td>
</tr>
<tr>
<td>Percichthyidae</td>
<td><em>Nannoperca oxleyana</em></td>
<td>spherical</td>
<td>0.98-1.10</td>
<td>no</td>
</tr>
<tr>
<td>Plotosidae</td>
<td><em>Tandanus tandanus</em></td>
<td>spherical</td>
<td>3.1-3.4</td>
<td>no</td>
</tr>
<tr>
<td>Pseudomugilidae</td>
<td><em>Pseudomugil mellis</em></td>
<td>spherical</td>
<td>1.26-1.64</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomugil signifer</em></td>
<td>spherical</td>
<td>1.13-1.8</td>
<td>yes</td>
</tr>
</tbody>
</table>
Morphology of the larvae of *N. oxleyana* is similar to that of other percichthyids described from Australia (Brown and Neira 1998, Trnski *et al.* 2005). There is an absence of larval specialisations. Head spination is limited to an opercular spine whereas most other described percichthyids also develop weak to moderate preopercular spines during, or shortly after, notochord flexion. *Nannoperca oxleyana* can be distinguished from other percichthyids by a combination of morphometrics, meristics and pigmentation pattern. In particular, the ratio of ED:SnL is greater than 200% throughout development (191% in only one wild specimen in the described series), which is higher than for other described percichthyids.

In some respects, the larvae of *N. oxleyana* more closely resemble those of other small to medium sized Australian percichthyids than the larger fishes of the genus *Maccullochella*. The larval lengths of *N. oxleyana* at hatching are comparable to those recorded for *N. australis* (3.2-3.9 mm), *N. vittata* (3.0-3.2 mm), *Macquaria ambiguа* (2.5-3.4 mm) and *M. novemaculeata* (2.5-3.5 mm), but are much smaller than those of *Maccullochella* spp. (5.0-9.0 mm), which hatch with a massive yolk (Shipway 1949; Lake 1967; Llewellyn 1974; Harris and Rowland 1996; Neira *et al.* 1998; Morgan and Beatty 2000; Pusey *et al.* 2004; Trnski *et al.* 2005). The larvae of species with similar lengths at hatching are also poorly developed at this time with unpigmented eyes and unformed mouths, while their lengths and ages at the commencement of exogenous feeding are also similar (lengths of 3.9-5.4 mm; ages of 3-8 days). Furthermore, in *N. oxleyana*, *N. australis*, *N. vittata*, *N. balstoni*, *Macquaria ambiguа*, and *Macquaria novemaculeata* the yolk is fully absorbed prior to notochord flexion, whereas in *Maccullochella peelii* and *Maccullochella macquariensis* the remnants of the yolk-sac are still visible in postflexion larvae (Llewellyn 1974; Harris and Rowland 1996; Rowland 1996; Brown and Neira 1998; Gill and Morgan 1998; Morgan and Beatty 2000; Pusey *et al.* 2004; Trnski *et al.* 2005).

Examination of larvae of *N. australis*, *N. obscura* and *N. variegata* revealed morphological and developmental similarities between these species and *N. oxleyana* (unpublished data). All species have melanophores distributed over the entire head and body with an internal line of pigment from the posterior of the eye to dorsally on the swim bladder and gut. *Nannoperca australis* and *N. obscura* also have 4-6 small patches of darker pigment on the dorsal midline of the trunk and tail, and the ventral midline of the tail, though these patches are absent in *N. variegata*. Although not sympatric, larvae of *N. oxleyana* can be distinguished from other pygmy perch species by meristics: *Nannoperca oxleyana* (D VI-VIII, 7-9; A III, 7-9; Vert 11-12+15-17=27-28), *Nannoperca australis* (D VI-IX, 7-10; A III, 6-9; Vert 12-13+16-18=29-30), *Nannatherina balstoni* (D VII-IX, 9-11; A III, 8-10; Vert 14+18=32), *Nannoperca*
Early development of *Nannoperca oxleyana*

*obscura* (D VIII-IX, 7-9; A III, 6-8; Vert 12+18=30), *Nannoperca variegata* (D VII-IX, 9-10; A III, 8-9; Vert 12-14+17-19=30-32) and *Nannoperca vittata* (D VII-IX, 8-11; A III, 6-9; Vert 11-13+17-18=29-31) (Whitley 1940; Kuiter and Allen 1986; Kuiter et al. 1996; Allen et al. 2002; and supplemented by x-rays of wild specimens housed in the Australian Museum fish collection).

*Nannatherina balstoni* obtains a larger maximum size than *N. oxleyana* of approximately 90 mm. It is the only other pygmy perch for which the larvae have been fully described (Gill and Morgan 1998). Ranges of body lengths for each stage of larval development of this species are: preflexion 4.9 – 6.6 mm, flexion 6.6-11.1 mm, postflexion 10.9-14.5 mm, and juveniles 14.1-23.2 mm. Apart from that of preflexion larvae, these ranges were larger at an equivalent stage than those for *N. oxleyana*. Squamation also commenced at a larger size in *N. balstoni* at 13 mm.

Melanophore distribution in the wild *N. oxleyana* larvae used in this description was sparser and lighter compared with the reared larvae. It is widely accepted that captive reared larvae are often heavier and have deeper bodies than wild caught specimens, display greater meristic variation and are frequently more heavily pigmented (Hunter 1984). Similarly, wild *N. oxleyana* larvae developed their meristic complements at a larger size than the reared series. These differences may be due to rearing conditions and also time of capture of the specimens.

It appears that four to five days post hatching represents an important period in the development of *N. oxleyana*. A combination of morphological, physiological and behavioural developments enabled the larvae to actively search for food and signalled the commencement of exogenous feeding. A similar timing and pattern of development was observed for *N. australis*, *N. vittata*, *M. ambigua* and *M. novemaculeata* (Llewellyn 1974; Battaglene and Talbot 1990; Rowland 1996; Morgan and Beatty 2000). Endogenous feeding was also still apparent at this time and growth rates proceeded to plateau over the following five to seven days as the last of the yolk was absorbed and larvae switched to full exogenous feeding. Trends in mortality rates may also reflect the transition from endogenous to exogenous feeding. On two occasions it was noted that the mean survival of 29 batches of larvae declined from 60.1% for larvae between the ages of 6 and 10 days to 10.4% for 11-14 day old larvae (J. Knight, unpublished data). Indeed, this transitional period is often one of high mortality (Blaxter 1969).

In this study, length proved to be a better measure of ontogenetic stage than age, at least after yolk-sac absorption. Ontogenetic states are often reached at uniform sizes, regardless of the
time taken to achieve them (Fuiman et al. 1998). Development rates are often influenced by environmental variables such as water quality, temperature, lighting regimes and food resources (Blaxter 1969; Fuiman et al. 1998). The rate of ontogenesis of eggs and larvae documented in this study may therefore simply reflect the rearing conditions in the laboratory. However, the potentially confounding effects of artificial conditions were minimised by utilising physico-chemical properties similar to waters supporting wild populations, by adopting the average photoperiod and water temperatures experienced by wild populations during the breeding season, and by providing a diet of zooplankton species recorded in the diets of wild larvae, juvenile and adult pygmy perch (Pen and Potter 1991; Pen et al. 1993; Arthington and Marshall 1993; Humphries 1995; Morgan et al. 1995; Arthington 1996; Gill and Morgan 1998). Research into the age and growth of *N. oxleyana* in the wild may assist in assessing if development and growth rates in captivity differ from those of wild fish.

*Nannoperca oxleyana* is one of Australia’s most endangered species and is the most threatened fish species to inhabit the coastal wallum ecosystems of mid-eastern Australia (NSW Department of Primary Industries 2005). Although the eggs of this species lacked filaments, in this study their slight adhesiveness facilitated attachment to the spawning mops until hatching. It is plausible that eggs spawned in the wild would attach to submerged riparian and aquatic vegetation in a similar way (Knight et al. 2007). Juveniles and adults occupy dense macrophyte beds and submerged riparian vegetation throughout the year (Arthington, 1996; Knight, 2000) and newly hatched, preflexion larvae have also been captured in these habitats (J. Knight, unpublished data). This habitat may therefore provide *N. oxleyana* with a refuge from predators and strong currents, and suitable feeding grounds throughout its entire life cycle (Pusey et al. 2004). Management practices, such as groundwater extraction, fire management, agriculture, dredging, channelisation, and urban development, that reduce or degrade available spawning, nursery and adult habitat pose a serious threat to this species (NSW Department of Primary Industries 2005). The conservation and recovery of *N. oxleyana* therefore depends largely on the maintenance of the remaining water bodies and associated wallum ecosystems.

**Acknowledgements**

This paper is from PhD research undertaken by J. Knight, and supported by NSW Department of Primary Industries (DPI), Southern Cross University (SCU), and the Australian Research Council. Our thanks to Consolidated Rutile Ltd for supplying the captive broodfish and to M. Hammer and A. King for providing larvae of other *Nannoperca* species as comparative
Early development of *Nannoperca oxleyana*

material. We also thank those DPI staff who assisted with animal husbandry, including G. Housefield, B. McCartin and N. Reed. B. Creese, A. Jordan and J. Leis provided comments on drafts of the manuscript. This research followed animal care and ethics protocol 3/14 approved by SCU.

**References**


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NSW Department of Primary Industries. 2005. *Oxleyan pygmy perch: Recovery Plan and Background Paper*. NSW Department of Primary Industries Fisheries Management Branch, NSW, Australia.


General Discussion and Conclusions

The biological diversity of the world is rapidly declining as a consequence of human activities. Human-induced extinction far exceeds the rate of evolution of new species and is considered comparable in size to the five mass extinctions revealed in the geological record (Frankham et al. 2002). The magnitude of this event and the realisation that Homo sapiens are one of many species impacted by ecological destruction has placed biological conservation firmly on the scientific and political agenda. The field of conservation biology, which aims to provide the principles and tools for maintaining biodiversity, is an increasingly important discipline correlated with the global effort to conserve and protect the natural environment and those species at risk of extinction (Meffe et al. 2006). In many cases information is urgently required to assist in the decision-making process for particular development or resource management proposals, or to determine recovery options for threatened species.

The research in this thesis has provided considerable new information on the endangered Oxleyan pygmy perch Nannoperca oxleyana Whitley. The central theme has been the examination of several fundamental biological attributes of this species and the ways in which environmental variables and anthropogenic impacts have influenced current patterns of distribution and abundance. This has been achieved through the application of a suite of applied conservation biology tools, including biogeography, population genetics, habitat ecology, reproductive biology and life history research, which have provided a number of recovery-based management and research principles for the conservation of N. oxleyana and for threatened fish species in general.

Integral to the conservation and management of threatened species is an understanding of the genetics and ecology of small, declining and fluctuating populations (Meffe and Vrijenhoek 1988; Burgman and Lindenmayer 1998; Frankham 2005; O’Grady et al. 2006). Hence, population conservation must be based firmly on an understanding of the links between the demographic processes of birth, death, and migration and the environment in which populations exist (Dunning et al. 2006). Population dynamics are also influenced by life history characteristics (e.g. size at first reproduction, larval development) as they play an important role in long-term population trends and the evolution of population characteristics (Dunning et al. 2006). This thesis has filled a number of knowledge gaps in the biology and early life history of N. oxleyana from which insights can be made into the dynamics of extant populations. An updated table of the life history and ecological characteristics of the species is provided in table 1.
Table 1. Life history and ecological characteristics of *Nannoperca oxleyana* updated from Table 2 in Chapter 1 with information from Knight *et al*. 2007 (Chapter 5) and Knight and Trnski (in press) (Chapter 6). * indicates J. Knight, unpublished data.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at maturity</td>
<td>In aquaria: 4-5 months</td>
</tr>
<tr>
<td>Minimum length of mature females</td>
<td>19 mm SL in Spitfire Ck, Qld; 19.9 mm near Evans Head, NSW</td>
</tr>
<tr>
<td>Minimum length of mature males</td>
<td>19mm SL in Spitfire Ck, Qld; 22.7 mm near Evans Head, NSW</td>
</tr>
<tr>
<td>Longevity</td>
<td>In wild: 6.5 years*; In aquaria: &gt; 6 years*</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>Unknown</td>
</tr>
<tr>
<td>Occurrence of ripe fish</td>
<td>In wild: all year; In aquaria: September - May</td>
</tr>
<tr>
<td>Peak spawning activity</td>
<td>In wild: October - December and February - April</td>
</tr>
<tr>
<td>Critical temperature for spawning</td>
<td>In wild and aquaria: water temperature $\geq 16.6^\circ$ C</td>
</tr>
<tr>
<td>Inducement to spawning</td>
<td>In wild and aquaria: water temp. $\geq 16.6^\circ$ C, day length $\geq 10.7$ hours</td>
</tr>
<tr>
<td>Mean gonadosomatic index of ripe fish</td>
<td>In wild: females: 3.3 - 4.1%; males: 0.6 – 0.7%</td>
</tr>
<tr>
<td></td>
<td>In aquaria: females: 4.2%; males: 0.7%</td>
</tr>
<tr>
<td>Mean batch fecundity (BF)</td>
<td>In wild and aquaria: 7.8 eggs/fish/day</td>
</tr>
<tr>
<td>Mean total (TF) and relative fecundity (RF)</td>
<td>In aquaria: TF = 1323 eggs/fish; RF = 589 eggs/g</td>
</tr>
<tr>
<td>Fecundity/Total Length (TL) relationships*</td>
<td>In aquaria: BF = -12.129 + 0.383TL; TF = -4522.7 + 0.383TL</td>
</tr>
<tr>
<td>Egg diameter</td>
<td>In aquaria at 25±1° C: 0.98 – 1.10 mm</td>
</tr>
<tr>
<td>Mean frequency of spawning</td>
<td>In aquaria: 57% of days in a 256 day spawning period</td>
</tr>
<tr>
<td>Oviposition and spawning site</td>
<td>In wild and aquaria: demersal, adhesive eggs attaching to aquatic veg.</td>
</tr>
<tr>
<td>Spawning migration</td>
<td>In wild: none observed</td>
</tr>
<tr>
<td>Parental care</td>
<td>In aquaria: guarding of spawning sites</td>
</tr>
<tr>
<td>Time to hatching/length at hatching</td>
<td>In aquaria at 25±1° C: 50 hours/2.8 – 3.4 mm Live Body Length (LBL)</td>
</tr>
<tr>
<td>Length/age at free swimming stage</td>
<td>In aquaria at 25±1° C: 3.9 – 4.6 mm LBL/5 days Post Hatch (PH)</td>
</tr>
<tr>
<td>Length*/age at loss of yolk sac</td>
<td>In aquaria at 25±1° C: 4.3 mm LBL/28 days PH</td>
</tr>
<tr>
<td>Length/age at first feeding</td>
<td>In aquaria at 25±1° C: 3.6 – 3.4 mm LBL/5 days PH</td>
</tr>
<tr>
<td>Length/age at metamorphosis into juveniles</td>
<td>In aquaria at 25±1° C: $\geq$9.6 mm LBL/70 days PH</td>
</tr>
<tr>
<td>Growth rate</td>
<td>In wild: increased from 14 mm to $\geq$ 28 mm TL over a one year period</td>
</tr>
<tr>
<td></td>
<td>In aquaria at 25±1° C: increased from 2.8 to 20.6 mm LBL in 6 months</td>
</tr>
<tr>
<td>Trophic guild and dietary composition</td>
<td>Microphagic carnivore consuming prey $\leq$ 5-6 mm including zooplankton, aquatic insects, atyid shrimps, terrestrial arthropods, and flying aquatic insects</td>
</tr>
<tr>
<td>Common co-occurring native fish</td>
<td>Firetailed gudgeon (<em>Hypseleotris galii</em>), empire gudgeon (<em>Hypseleotris compressa</em>), striped gudgeon (<em>Gobiomorphus australis</em>), softspined rainbowfish (<em>Rhadinocentrus ornatus</em>), Duboulay’s rainbowfish (<em>Melanotaenia duboulayi</em>), honey blue-eye (<em>Pseudomugil mellis</em>)</td>
</tr>
<tr>
<td>Negative interactions with native fish</td>
<td>In wild: none observed</td>
</tr>
</tbody>
</table>
The available information supports the notion by Knight (2000) that *N. oxleyana* has an *r*-selected life strategy. *r*-selected species are characterised by a high growth rate, early maturity at a small size, high reproductive output, a high mortality rate and, as a consequence, fluctuating population sizes driven more by environmental conditions than demographic forces (Wootten 1992; Odum 1997). This strategy is most evident in those populations inhabiting the source and sink floodplain habitats of the Richmond and Clarence Rivers of coastal NSW (Knight *et al.*, 2007 [Chapter 5]; Chapter 4).

*Nannoperca oxleyana* is further characterised by specific habitat requirements (Knight and Arthington 2008 [Chapter 3]), which is more consistent with *K*-selected species. This combination of *r*- and *K*-selected traits suggests that while large population sizes are achievable in undisturbed habitats, any reduction in the quality of habitat or variation in the environment occupied by the species may result in substantial decreases in population sizes. Dramatic population fluctuations linked to patterns of rainfall and drought have indeed been documented in the wild (Knight 2000; Knight *et al.* 2007 [Chapter 5]) and there is evidence to suggest that anthropogenic habitat disturbances causes localised population declines and extinctions (Arthington and Marshall 1993; Arthington 1996; Knight 2000, in press; Knight and Butler 2004; NSW DPI 2005; Knight and Arthington 2008 [Chapter 3]; Chapter 4). Given that population densities appear to be governed less by demographic processes and more by independent environmental uncertainty, management initiatives focused on maintaining and rehabilitating habitats, dispersal corridors and environmental conditions supporting *N. oxleyana* are likely to be more beneficial to the conservation of this species than activities such as stock enhancement aimed at increasing recruitment.

Understanding the present day distribution and abundance patterns of *N. oxleyana* requires not only an understanding of its biology and ecology but also of historical patterns of biogeography inferred from landscape evolution patterns in which the species evolved (Keenan 1994; Unmack 2001; Ponniah and Hughes 2006; Page and Hughes 2007). For example, genetic variation in the species is thought to be partly a consequence of recent, historical gene flow along the mid-east Australian coast (Chapter 4). This pattern is likely to be a function of fluvial evolution patterns that facilitated dispersal. Further insights into the biogeography of *N. oxleyana* and its relevance to the conservation of this species are discussed below.
General Discussion and Conclusions

It is currently hypothesised that most of Australia’s freshwater fish fauna including percichthyids evolved from marine ancestors trapped in an ancient retreating inland sea (Jerry et al. 2001; Allen et al. 2002, 2005). This sea entered Australia during Cretaceous times over 120 million years ago (mya) and fluctuated in relation to global sea level changes and geological events for nearly 25 million years. Approximately 90 mya, ocean rifting resulted in the uplifting of the Great Dividing Range, thereby separating the inland Murray-Darling Basin from the east coast drainages (Ollier 1995). Despite this apparent barrier to fish dispersal, high faunal similarity currently exists between the inland basin and drainages east of the Great Dividing Range. In particular, nine species co-occur in both the Murray-Darling Basin and the Clarence River, while the former basin shares eleven species with the Fitzroy drainage in central Queensland (Unmack 2001). There are a number of genera including *Nannoperca* whose distributions also straddle the divide (Allen et al. 2002). These trends have been attributed to either the geological process of stream capture or simply areas of lesser elevation in the Great Dividing Range, which under certain climatic or geological conditions such as flooding and volcanism may have allowed fish to cross the mountain range (Musyl and Keenan 1992; Rowland 1993; McGlashan and Hughes 2001; Unmack 2001).

Based on geomorphologic and electrophoretic research, it is conceivable that speciation within the genus *Nannoperca* may have resulted from the separation of the inland and east coast drainages. The entire distributional range of *N. oxleyana* falls within the expansive Clarence-Moreton Basin, which was once drained primarily by the Clarence River (Haworth and Ollier 1992). This river initially drained the Clarence-Moreton Basin westward into the Condamine River of the Murray-Darling Basin, but creation of the Great Dividing Range split these two rivers approximately 65-25mya and caused the Clarence to flow eastward towards the coast (Haworth and Ollier 1992) (Figure 1). It is possible that *Nannoperca* may have been separated by and diverged subsequent to this vicariant event as it is considered one of the most derived genera within the Percichthyidae (Johnson, 1984; Jerry et al., 2001). This divergence between *N. oxleyana* and extant relative species is thought to have occurred in the distant past, as far back as tens of millions of years (M. Hammer, University of Adelaide, South Australia, pers. comm.).
Figure 1. Geological events attributed to the distribution of *Nannoperca oxleyana*. Extent of Mount Warning shield volcano is taken from Graham (2004a).
Alternatively, *N. oxleyana*, a congener, or an ancestor may have more recently crossed the divide at areas of lesser elevation in the Great Dividing Range or by the geological process of stream capture. For example, the low point between the Fitzroy and Burnett drainages (Unmack, 2001) may have facilitated the dispersal of fish into this area and then southward into the adjacent Mary River catchment where present day populations of *N. oxleyana* exist (Figure 1). Likewise, the headwaters of the Clarence River are one of the few places with elevations low enough to allow fish to cross the divide from the Murray Darling Basin to the east coast (Unmack, 2001). However, recent genetic research suggests the possibility that dispersal may have occurred in the opposite direction, with coastal populations of southern purple spotted gudgeon *Mogurnda adspersa* (Castelnau) (Eleotridae), eel-tailed catfish *Tandanus tandanus* (Mitchell) (Plotosidae), golden perch *Macquaria ambigua* (Richardson) (Percichthyiidae) and Macquarie perch *Macquaria australasica* Cuvier (Percichthyiidae) all apparently ancestral to populations in the Murray-Darling Basin (Faulks et al., 2008; Jerry, 2008; L. Faulks, Macquarie University, pers. comm.). These findings contradict the ‘inland sea hypothesis’ and given the evolution of the Clarence River, lead to the possibility that the genus *Nannoperca* may have also evolved in a similar way.

Given the current fragmentation of Queensland and NSW populations of *N. oxleyana* it is possible that the species never dispersed into those catchments within the distribution gap or was once present there but became locally extinct via natural or human interventions. The species absence from most of the Richmond River and the Brunswick, Tweed and Logan-Albert Rivers coincides with the presence of the Mount Warning shield volcano (Figure 1). This volcano formed 23.5 to 20.5 mya during the Miocene Epoch and like many shield volcanos, was responsible for the formation and super-imposition of radial drainage in the vicinity and major drainage disruption in adjacent catchments (Ollier, 1995; Graham, 2004a). It particularly altered the Brunswick and Tweed Rivers but its influence spread over a wider area from the Logan River southward to most of the Richmond River. Miocene volcanism also restricted drainage to the Clarence River, confining it largely to the south-eastern part of the Clarence-Moreton Basin (Haworth and Ollier 1992). These processes may have fragmented the distribution of *N. oxleyana* through extinction or impeded dispersal into these catchments.

A correlation also exists between the occurrence of the species and areas in northern NSW and southern Queensland inundated by marine transgressions during more recent interglacial periods. While its distribution coincides with large sand plains formed when sea levels were five or six metres higher than present approximately 120 000 to 130 000 years ago, the
species is conspicuously absent from areas inundated 6 500 to 4 000 years ago, when sea levels were approximately one to one and a half metres higher than present (Lambeck and Nakada 1990; Graham 2004b; Knight and Arthington 2008 [Chapter 3]; Knight in press). Alternatively, lower sea levels as recently as 8 000 years ago were likely to have allowed *N. oxleyana* to disperse northward and southward to colonise other drainage systems (Chapter 4). During this period however, the radial drainages associated with Mount Warning may have possibly remained separate from adjacent systems thereby impeding colonisation of this area.

Ecological requirements and tolerances are also important when considering biogeographic patterns. Extinction probability is likely to be higher for a habitat specialist like *N. oxleyana*, particularly if dispersal is impeded (Unmack 2001). Human activities appear to have had a significant influence on the species presence/absence patterns and may have been responsible for its current distributional limits and gap within this distribution. Limited dispersal opportunities for populations within discrete drainages or fragmented environments make repopulating areas following disturbances difficult (Noss *et al.* 2006). Habitat destruction, degradation and fragmentation within the Tweed and Brunswick catchments, which lack the large coastal floodplains amenable to dispersal such as those in Broadwater and Bundjalung National Park, may have caused the localised extinction of *N. oxleyana* in this area. A combination of past volcanism, recent marine transgressions and/or anthropogenic impacts is also plausible, as are climatic conditions such as droughts, which may have caused the localised extinction of populations with limited dispersal opportunities (Unmack 2001; Magoulick and Kobza 2003; Matthews and Marsh-Matthevs 2003).

The majority of extant populations of *N. oxleyana* are relatively secure within National Park estate. However, a number of anthropogenic impacts that threaten *N. oxleyana* populations outside of reserves are also operating within these areas. These include the degradation of downstream habitats outside of reserves (i.e. formation of sink habitats), periodic channelisation of streams, disruption to fish passage, water pollution and riparian degradation associated with public access, the introduction of alien and translocated species, and encroaching urban, rural and agricultural development (Pusey *et al.* 2004; NSW DPI 2005, Knight in press).

One of the greatest impacts on this species is thought to have been the environmental destruction associated with sand mining during the 20th century (NSW DPI 2005). Ironically its legacy of deep artificial dredge ponds scattered over the coastal floodplains of northern NSW have since provided drought refuge habitat for the local aquatic fauna including *N.
oxleyana. The severity of the drought experienced during this study has been intrinsically linked to anthropogenic climate change (Karoly et al. 2003), which has recently been recognised as a significant threat to freshwater fishes (Matthews and Marsh-Matthews; 2003; Pusey et al. 2004). Likewise, long-term climatic variability has been positively related to sea level change along the Australian coast (Lambeck and Nakada 1990; Kershaw et al. 2003), with human-induced global warming and associated sea level rises conceivably posing a major threat to the coastal freshwater environments inhabited by N. oxleyana.

The contemporary distribution and abundance patterns of populations of N. oxleyana are therefore likely to be a construct of landscape evolution, climatic conditions and recent human influences at a variety of temporal and spatial scales. It is evident that the recovery of this species is dependent upon the protection of the particular macro-, meso- and microhabitat features shown to be associated with healthy extant populations. One such mechanism facilitating the formal protection of all water bodies supporting the species both inside and outside protected areas includes a declaration of Critical Habitat under state and Commonwealth legislation. Protection of N. oxleyana habitat also requires an understanding of the processes that create and maintain habitat structure and variability within the broader landscape of the coastal lowlands and the wallum ecosystem. Hence, management strategies should be focused on maintaining environmental drivers such as natural climatic and river flow regimes, riparian vegetation and nutrient dynamics in dystrophic ecosystems. In addition, it is necessary to understand the processes that govern the dispersal of the species along connectivity pathways in floodplain rivers, as well as identifying the main breeding populations, sources of colonists and possible movement pathways into individual drainages and isolated water bodies. In this regard, population genetics research using sensitive molecular techniques such as microsatellite markers may elucidate patterns of contemporary gene flow and dispersal among drainages as well as inform efforts to increase within-population genetic diversity of remnant populations suffering the effects of small population size (Chapter 4). This thesis has laid the foundation for these research initiatives.

Ultimately, the survival of N. oxleyana and indeed the world’s biodiversity depends largely upon the ability of human beings to stem the tide of anthropogenic climate change, excessive resource use and environmental destruction, and to effectively maintain the natural processes governing genetic diversity, diversity among organisms and ecological systems. The multidisciplinary approach provided by conservation biology, such as the one adopted in this thesis, provides an avenue through which this can be achieved.
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